



THE UNIVERSITY OF QUEENSLAND
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Wideband Absorbance Measures in Neonates and Young Infants

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ABSTRACT

Evaluation of middle ear status in young infants is a challenge in both screening and diagnostic contexts due to a lack of valid and objective tools of middle ear assessment (Kei & Zhao, 2012). The standard tools used to determine the middle ear status in older children are neither efficient nor accurate in evaluating young infants.

Wideband absorbance (WBA) is recommended as a tool for middle ear assessment in young infants due to its time efficiency, reliability, objectivity and ability to provide clinical information over a range of frequencies. Despite its clinical application in the assessment of middle ear function in older children, investigation into the use of WBA with young infants has been inadequate. Development of normative data and evaluation of WBA in this population have been limited. The limitation of using the distortion product otoacoustic emissions (DPOAE) test as a reference standard for evaluation of middle ear status has been acknowledged. Hence, further consideration must be afforded to evaluation of WBA using more robust reference standards in young infants.

Despite the high prevalence of conductive hearing loss in Australian Aboriginal children, limited research has occurred into the investigation of middle ear function in the neonatal period. Hence the present study explored the development of normative data and evaluation of test performance of WBA, developmental and pathologic effects in middle ear function and the feasibility of using WBA as an adjunct tool in newborn hearing screening (NHS) programs in an Australian context.

The present research study aimed to: (1) determine the prevalence of middle ear dysfunction and conductive hearing loss in neonates referred through a NHS program in Australia (2) establish normative WBA measures in healthy neonates with normal middle ear function (3) compare test performance of WBA against nine reference standards (4) compare WBA between Australian Aboriginal and Caucasian neonates and (5) compare WBA in healthy newborns and infants aged 1, 2, 4 and 6 months.

A chart review of 234 infants referred from a NHS program in North Queensland was conducted. Further, 204 neonates were tested using automated auditory brainstem response (AABR), high frequency tympanometry (HFT), acoustic stapedial reflex (ASR), transient evoked otoacoustic emissions (TEOAE), DPOAE and WBA. The neonates were tested prior to their discharge from the hospital. In addition, a total of 36, 30, 33 and 30 infants were seen at 1, 2, 4, and 6 months of age, respectively. These infants were tested using HFT, DPOAE and WBA.

The results revealed that conductive hearing loss was common among infants referred through NHS. Australian Aboriginal infants had significantly higher rates of middle ear pathology and conductive hearing loss at birth and showed poor resolution of middle ear pathology over time compared to non-Aboriginal infants. Use of a test of middle ear function (eg. WBA) as an adjunct to the screening tool to facilitate management and prioritisation of infants for further testing was recommended (Chapter Two). Normative ambient pressure WBA data were established for 66 neonates who passed a test battery of AABR, HFT, ASR, TEOAE and DPOAE tests. There was a significant difference in WBA across frequencies from 250 to 8000 Hz (Chapter Three). Test performance of WBA was compared across four single tests and five test battery reference standards in 192 neonates. The test performance of WBA against the test battery reference standards was better than that against single test reference standards (Chapter Four). Despite equal pass rates as determined by a test battery of HFT and DPOAE, the WBA of Aboriginal neonates who passed the test battery was significantly lower suggesting that Aboriginal neonates had more significant outer/middle ear conditions than Caucasian neonates. WBA appeared to be more sensitive to middle ear status than test battery comprising HFT and DPOAE (Chapter Five). In a cross sectional study of infants, developmental effects were evident during the first six months of life, with WBA reducing with age. Although data from the study could be used as reference standard for detecting middle ear disorders, further development of age-specific normative WBA was recommended (Chapter Six). Overall, the present research study demonstrated WBA to be a feasible tool for evaluation of middle ear function in neonates and young infants (Chapters Three, Four, Five).

In conclusion, this thesis has evaluated WBA in neonates and young infants and has enhanced the minimal literature available concerning normative data, test performance, developmental changes and application in targeted groups such as Australian Aboriginal infants where the prevalence of otitis media is very high. The normative data developed in the study may be used as a reference for objective evaluation of the sound conduction pathways (outer and middle ear) in neonates and young infants. Ultimately, this thesis contributes to the application of WBA as a clinical tool in the assessment of middle ear function during screening or diagnostic assessment of neonates and young infants.

DECLARATION BY AUTHOR

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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PUBLICATIONS DURING CANDIDATURE

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Aithal, V.	Obtained ethical consent (100%) Designed experiment (20%) Data collection (20%)
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Australian Aboriginal, conductive hearing loss, middle ear dysfunction, neonates, wideband absorbance, wideband tympanogram, young infants.

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List of Abbreviations Used in the Thesis

AABR	Automated Auditory Brainstem Response
AAI	Absorbance Area Indices
ABG	Air Bone Gap
ABR	Auditory Brainstem Response
AC	Air Conduction
ANOVA	Analysis Of Variance
APGAR	Appearance, Pulse, Grimace, Activity and Respiration
AROC	Area under the Receiver Operating characteristic Curve
ASR	Acoustic Stapedial Reflex
ASSR	Auditory Steady State evoked Response
ATSI	Aboriginal and Torres Strait Islander
B	Susceptance
BC	Bone Conduction
CI	Confidence Interval
cm	Centi meters
daPa	Deca Pascals
dB	Decibel
dBA	A Weighted Decibel
DPOAE	Distortion Product Otoacoustic Emissions
ENT	Ear, Nose and Throat
G	Conductance
g	Grams
G-G	Greenhouse and Geisser
h	Hours
hr	Hours
HFT	High Frequency Tympanometry
HL	Hearing level
Hz	Hertz
kHz	Kilo-Hertz
MEE	Middle Ear Effusion
MEMR	Middle Ear Muscle Reflex

Min	Minutes
ml	Milli Litres
mmho	Milli Mhos
mo	Months
MRL	Minimum Response Level
μs	Micro Seconds
msec	Milli Seconds
n	Number
nHL	Normalised Hearing Level
NHS	Newborn Hearing Screening
NICU	Neonatal Intensive Care Unit
OAE	Otoacoustic Emissions
OM	Otitis Media
OME	Otitis Media with Effusion
pkSPL	Peak Sound Pressure Level
RMS	Root Mean Squared
ROC	Receiver Operating Characteristics
s	Seconds
SD	Standard Deviation
SEM	Standard Error of Mean
SNR	Signal to Noise Ratio
SPL	Sound Pressure Level
TEOAE	Transient Evoked Otoacoustic Emissions
TPP	Tympanometric Peak Pressure
UNHS	Universal Newborn Hearing Screening
VRA	Visual Reinforcement Audiometry
WAI	Wideband Acoustic Immittance
WBA	Wideband Absorbance
WBR	Wideband Reflectance
WBT	Wideband Tympanometry
Y	Admittance
YBC	Baseline compensated static admittance
YCC200	Component compensated admittance at +200 daPa
YCC-400	Static admittance compensated for two components at -400 daPa

Chapter One: Introduction

1.1 Organisation of Thesis

The thesis is presented as a series of published articles, manuscripts accepted for publication and currently under review for publication. Each article is presented as a separate chapter and an introduction to the research study is provided to illustrate the relevance of the article to the research topic. Implications and recommendations arising from this research are discussed in the last chapter. Apart from the first and last chapters, this thesis consists of articles that have been written as stand-alone journal publications. Hence, there will be some repetition of concepts and citations across the chapters.

1.2 Introduction

Universal newborn hearing screening (UNHS) programs aim to detect congenital permanent hearing loss in newborns. However, the commonly used instruments, automated auditory brainstem response (AABR) and otoacoustic emission (OAE) tests, cannot distinguish between conductive and sensorineural hearing impairments. A “refer” outcome due to the presence of outer and middle ear dysfunction is often classified as a false positive result. Management of false positive results is an important issue in UNHS programs (Boone, Bower & Martin, 2005; Doyle, Rodgers, Fujikawa & Newman, 2000; Sanford et al., 2009). Apart from increased false positive referrals, transient middle ear conditions such as middle ear effusion (MEE) can also delay definitive diagnosis of sensorineural hearing loss (Boone et al., 2005; Vartiainen, 2000).

Assessment of middle ear function in neonates and young infants is challenging due to a lack of valid, objective tools of middle ear assessment in this population (Kei & Zhao, 2012). Several studies conducted in the last decade have recommended 1000 Hz tympanometry for the assessment of middle ear function in infants from birth to six months of age (Calandruccio, Fitzgerald & Prieve, 2006; Kei et al., 2003; Margolis, Bass-Ringdahl, Hanks, Holte & Zapala, 2003; Mazlan, Kei & Hickson, 2009; Swanepoel et al., 2007). However, recent studies by Sanford et al. (2009) and Hunter, Feeney, Miller, Jeng, and Bohning (2010) have shown 1000 Hz

tympanometry to be less accurate in predicting the effects of sound conduction deficits. Similarly, other measures such as acoustic stapedial reflex (ASR) and automated auditory brainstem response (AABR) have not had much success in detecting subtle middle ear dysfunction in this population (Hunter, Prieve, Kei & Sanford, 2013; Stapell, 2011). Due to these constraints, there is a need for sensitive, specific and user friendly screening tools that permit better interpretation of middle ear status in infants and young children (Hunter et al., 2013).

Wideband absorbance (WBA), an emerging technique, has been suggested as an alternate tool for the assessment of middle ear disorders in infants and children (Hunter et al., 2010; Keefe, Bulen, Arehart & Burns, 1993; Keefe et al., 2000; Sanford et al., 2009). Although WBA is reported to be sensitive to various middle ear disorders in children (Beers, Shahnaz, Westerberg & Kozak, 2010; Ellison et al., 2012; Hunter, Bagger-Sjöbäck, & Lundberg 2008a; Jeng, Levitt, Lee & Gravel, 1999; Keefe & Simmons, 2003; Keefe, Sanford, Ellison, Fitzpatrick & Gorga, 2012; Piskorski, Keefe, Simmons & Gorga, 1999) and adults (Allen, Jeng & Levitt, 2005; Feeney, Keefe & Maryott, 2003; Keefe et al., 1993; Keefe & Simmons, 2003; Shahnaz et al., 2009), there is limited research on WBA in neonates and young infants. Available research suggests that WBA can predict OAE outcomes more accurately than 1000 Hz tympanometry (Hunter et al., 2010; Sanford et al., 2009). Inclusion of WBA in newborn hearing screening (NHS) programs has shown to decrease the false positive rates from 5% to 1% (Keefe, Zhao, Neely, Gorga & Vohr, 2003b). Consequently, WBA has been recommended as an adjunct tool in UNHS programs (Feeney and Sanford, 2012; Hunter et al., 2010; Merchant, Horton & Voss, 2010; Sanford et al., 2009; Vander Werff, Prieve & Georgantas, 2007; Werner, Levi & Keefe, 2010).

Apart from the limited normative WBA data available with neonates, evaluating the test performance of WBA is challenging. While tone burst air and bone conduction ABR may be used as a surrogate gold standard for detecting conductive hearing loss in United Kingdom, Canada and United States of America, this threshold ABR measure is time consuming and is done diagnostically at some point later in time rather than in newborn hearing screening programs. Most studies circumvent this issue by using either distortion product otoacoustic emission (DPOAE) or high

frequency (1000 Hz) tympanometry (HFT) as a reference standard for determining the status of the outer and middle ear (Sangster, 2011). However, DPOAE or HFT alone may not accurately identify minor middle ear pathologies (Hunter et al, 2010; Kemp, 2002; Sanford et al., 2009) and, hence, may not serve as an ideal reference standard. Therefore, the use of DPOAE or HFT as a reference standard represents a significant shortcoming in assessing outer and middle ear function in neonates.

In order to evaluate the efficacy of WBA as a clinical tool in neonates and young infants, it is important to use a better reference standard than current standards (Hunter et al., 2013). Since a reference standard based on the outcomes of a surgical procedure cannot be used in view of ethical considerations, an optimal reference standard based on the outcomes of a combination of tests such as HFT, DPOAE and transient evoked otoacoustic emission (TEOAE) may be used.

Despite the introduction of NHS across Australia, the use of WBA in evaluating the conductive pathways (outer and middle ear) in neonates and young infants has not been systematically investigated. Earlier attempts to determine the prevalence of conductive conditions in neonates (Caucasian and Aboriginal descent) using traditional techniques such as 226 Hz tympanometry and OAEs found that conductive conditions are more prevalent in Aboriginal than non-Aboriginal neonates (Boswell & Nienhuys, 1995, 1996; Lehmann et al. 2008). These prevalence rates, however, need updating using a new and more accurate technique such as WBA.

1.3 Chapter Synopsis

The aim of the present chapter is to review the application of currently available audiological and non-audiological tests in the assessment of outer and middle ear function in neonates and young infants. In this chapter, the literature is discussed in relation to the issue of false positive referrals in UNHS programs, conductive hearing loss and middle ear pathology in neonates and young infants, otitis media (OM), as well as conductive hearing loss in Australian Aboriginal and Caucasian infants and children. In particular, this chapter will introduce a new technology, wideband absorbance (WBA) as an alternate measure of outer and middle ear function in this population. The application of WBA in neonates, infants, children

and adults are reviewed. Finally, the rationale for the current study is discussed and specific aims and major hypotheses are outlined.

1.4 Universal Newborn Hearing Screening Program – Identification of Sensorineural vs Conductive Hearing Loss

The primary goal of any early hearing detection program is to correctly differentiate ears with hearing loss from healthy ears (Keefe et al., 2000; Sanford et al., 2009). Currently, UNHS programs use OAE and/or AABR for hearing screening. The limitation of both screening technologies is that the outcomes are influenced by both outer/middle and inner ear conditions. In healthy neonates screened for sensorineural hearing loss using either technology, a common finding is a false positive response due to transient ear canal and/or middle ear obstruction (Allen et al., 2005; Doyle, Burggraaff, Fujikawa, Kim & Macarthur, 1997; El-Rafaie, Parker, & Bamford, 1996; Gorga, Preissler, Simmons & Hoover, 2001; Keefe et al., 2000; Orlando & Prieve, 1998; Thornton, Kimm, Kennedy, & Cafarelli-dees, 1993; Watkin & Baldwin, 1999; White, Vohr & Beherns, 1993). For example, the prevalence of bilateral sensorineural hearing loss in neonates has been estimated to be between one and two per 1000 (Aidan, Avan, & Bonfils, 1999; Australian Hearing, 2005; Cunningham & Cox, 2003; Feinmessenger, Tell, & Levi, 1982; Kemper & Downs, 2000; Thringer, Kankkunen, Linden & Niklasson, 1984). In contrast, it has been reported that conductive hearing losses due to congenital MEE or more permanent outer and middle ear conditions occur at a rate thirty times greater than sensorineural hearing losses in young infants (Gorga et al., 2001; Orlando & Prieve, 1998; White et al., 1993).

White et al. (1993) reported that 17/1000 well babies and 36/1000 babies in the newborn intensive care unit (NICU) had a conductive hearing loss. Boone et al. (2005) noted that MEE may contribute up to 67% of the false positive newborn hearing screens. Boudewyns et al. (2011) reported that 53.5% of 152 infants, who were referred because of unilateral or bilateral failure on AABR screening, had MEE. The above studies demonstrate that middle ear conditions are more prevalent than sensorineural conditions in neonates.

Apart from increased referral rates, middle ear pathology can also delay the diagnosis of permanent hearing loss due to ambiguous results (Boone et al., 2005; Vartiainen, 2000). In order to reduce false positive rates and improve identification of infants with permanent hearing loss in UNHS programs, it is important to be able to discriminate ears with transient conductive problems from ears with a sensorineural hearing loss. Hence, there is a need for a screening tool that is sensitive, specific and user-friendly that can permit better identification of outer and middle ear status and can be used as an adjunct to OAE/ABR screening in neonates (Gravel et al., 2005; Hunter et al., 2013).

1.5 External/Middle Ear Pathology and Conductive Hearing Loss in Neonates

As mentioned previously, transient conductive hearing loss due to MEE and/or occluded ear canals accounts for the majority of referrals in a NHS program (Doyle et al., 2000; Doyle, Kong, Strobel, Dallaire & Ray, 2004; Keefe et al., 2000; Kok, vanzanten, & Brocaar, 1992; Rosenfeld et al., 2004; Takahara, Sando, Hashida, & Shibahara, 1986; Thornton et al., 1993). For instance, external canal obstruction due to vernix caseosa, a waxy substance that covers the skin of the neonate, is reported to be related to increased failure rates in NHS. In a study of 82 ears of neonates with a mean age of 43 hours, Chang, Vohr, Norton, and Lekas (1993) found that 76% of ears passed OAE before otoscopic examination and that the pass rate improved to 91% following vernix removal, thus, attributing 15% of the failure rate to external canal obstruction. Doyle et al. (1997) studied 400 ears of healthy neonates aged 5 to 48 hours and found that cleaning of vernix resulted in a 5% improvement in the ABR pass results from 91 to 96% and a 10.5% improvement in the OAE pass results from 58.5 to 69%.

Other studies have used otoscopic examination to determine the prevalence of vernix caseosa in the external ear canal and the degree of obstruction it creates. Balkany, Berman, Simmons, and Jafek (1978) studied 50 infants less than 24 hours of age and reported that all infants had at least partial obstruction of the ear canal. Cavanaugh (1987) found that on day 1, 56% of the ear canals were obscured and this reduced to 19% by day 3. On otoscopic examination of 400 ears of infants aged 5 to 48 hours, Doyle et al. (1997) found vernix obscuring the view of the tympanic membrane in 53 (13%) ears.

In addition to vernix, which can influence the outcomes of NHS in the first few days of life, residual mesenchyme and amniotic fluid have also been reported to contribute to conductive conditions in neonates. Temporal bone studies have demonstrated that the condition of the middle ear in neonates is seldom pristine (Benner, 1940; Buch & Jorgensen, 1964; deSa, 1973, 1983; Hemsath, 1936) with MEE present in up to 50% of ears (deSa, 1973; Eavey, 1993). The middle ear and antrum of a neonate have been reported to contain some amniotic fluid or residual mesenchyme and its cellular content. Studies have shown that in the early stages of foetal development, the middle ear is filled with mesenchyme, which resolves between 8 foetal months to 13 postnatal months (Guggenheim, Clements, & Schlesinger, 1956; Jaisinghani, Paparella, Schachern, & Le, 1999; Piza, Gonzalez, Northrop, & Eavey, 1989; Takahara et al., 1986).

Amniotic fluid contents aspirated into the middle ear have often been reported to contribute to MEE and conductive hearing loss in newborns (deSa, 1973; Northrop, Piza, Karmody, & Eavey, 1999; Roberts et al., 1995). The volume of aspirated amniotic fluid has been reported to vary markedly from a very scant amount to a sizeable inoculum that fills up a substantial portion of the middle ear and antral space (Northrop, Piza, & Eavey, 1986; Piza et al., 1989). The aspirated amniotic debris, instead of being cleared rapidly from the airways and the middle ears, may persist for several days (deSa, 1973). Histologic observations have shown that this material induces a significant inflammatory response of a foreign body giant cell reaction that produces a large volume of granulation tissue as well as advanced inflammatory responses resulting in extensive damage to the major attic compartments and under-pneumatisation of the mastoid (deSa, 1973; Eavey, Camacho, & Northrop, 1992; Palva, Northrop, & Ramsay, 2001; Piza et al., 1989; Ramsay, Palva, & Northrop, 2001).

Therefore, it is likely that during the immediate postnatal period, a conductive hearing loss may be present due to this amniotic fluid, followed by improvement in hearing as this fluid is cleared (Priner, Freeman, Perez, & Sohmer, 2003). The majority of the studies of MEE in neonates have been histopathological and temporal bone studies. However, the prevalence of middle ear pathology and conductive

hearing loss (presumably due to MEE) has been derived from the NHS results. For instance, Stuart, Yang and Green (1994) attributed an ABR air bone gap (ABG) of more than 12 dB within the first 48 hours after birth to residual amniotic fluid in the middle ear. Kok et al. (1992) reported the inability to record OAEs in 50% of neonatal ears 3 to 51 hours after birth, while 24 hours later OAEs could be recorded in all ears. They explained that this improvement could be due to the clearance of fluid from the middle ear during that period. Using a combination of otoscopy, OAE and ABR in their study of 200 neonates aged 5 to 48 hours, Doyle et al. (1997) found the prevalence of MEE to be 9%. Infants with decreased tympanic membrane mobility by pneumatic otoscopy had failure rates of 50% and 62.5% for ABR and OAE, respectively, compared with failure rates of 11.5% and 21% for the entire sample. Boone et al. (2005) identified MEE in 64.5% of 76 infants referred for diagnostic evaluation through NHS and attributed it to residual amniotic fluid. Using a combination of otoscopy, acoustic reflex measurements and tympanometry, Roberts et al. (1995) reported MEE to be present in all 68 babies examined in the first three hours of life. By the third day, MEE had resolved in 73% of ears by otoscopy, 88% by acoustic reflex measurements and 92% by tympanometry. MEE is, therefore, a condition that can affect hearing in neonates and in turn influence the outcomes of UNHS programs.

In summary, vernix in the ear canal and mesenchyme or amniotic fluid in the middle ear have been reported to be the common causes of false positive results during NHS. In order to reduce the false positive rates, there is an urgent need to assess the conductive system at the time of screening in order to differentiate the ears with transient outer and middle ear pathologies from the ears with a sensorineural hearing loss.

1.6 Otitis media and Conductive Hearing Loss in Australian Infants

Otitis media (OM) is a generic term for inflammation within the middle ear cleft, while MEE denotes a liquid in the middle ear cleft regardless of etiology (Gates, 1996). Australian Aboriginal infants and children are reported to have a high prevalence of OM compared to Caucasian infants and children. However, there has been limited research into the ear health status of Aboriginal infants at birth.

1.6.1. Aboriginal infants

Several prospective otoscopic and audiological studies have documented a very high prevalence of OM in young Aboriginal infants. Rebgetz, Trennery, Powers, and Mathews (1989) showed that the tympanic membrane perforations began in early infancy, from as early as eight weeks. Douglas and Powers (1989) found that, by one year, up to two thirds of Aboriginal infants had at least one perforated ear drum. Peak incidences of first ear drum perforation occurred at around 18 weeks and at 50 weeks. Foreman, Boswell, and Mathews (1992) reported that 16% of Aboriginal infants aged 4 to 6 months and 30% of older children in three Northern Territory communities had perforated tympanic membranes. In another study, Foreman (1987) found that of 425 ears examined in Aboriginal infants and young children, only five ears (1.2%) were normal and 420 ears (98.8%) had evidence of abnormality. The prevalence of perforation was 11% at age 0 to 6 months, 43% at 7 to 12 months and 30% at 13 to 24 months. Discharge from ears commenced from three months of age with a maximum incidence of 50% in the 10 to 12 months age group. For those with intact ear drums in the youngest group, there was a high prevalence of otoscopic abnormalities: 20% were inflamed and 35% were dull at 0 to 6 months. While studies suggest genetic predisposition to ear infections as a contributing factor (Wiertsema and Leach, 2009), more research is required to substantiate this claim. Nevertheless, environmental factors such as high rates of cross infection due to overcrowding, poor hygiene and high rates of early bacterial colonisation have been reported to major causes of ear disease in Aboriginal neonates (Hill, 2012; Leach, Boswell, Asche, Nienhuys, & Mathews, 1994; Morris et al., 2009).

Despite the high prevalence of ear diseases in young Aboriginal children, there is limited research on outer and middle ear function during the neonatal period. In a longitudinal study of 22 Aboriginal infants, Boswell and Nienhuys (1995) showed that Aboriginal infants had middle ear aeration and normal hearing shortly after birth and otitis media with effusion (OME) or acute OM was diagnosed in 95% of these infants within six to eight weeks of birth. They also reported that once middle ear disease started early in life, it became persistent despite treatment. Lehmann et al. (2008) studied 100 Aboriginal children from birth to two years of age with routine check up by an ear, nose and throat (ENT) specialist thrice a year and hearing assessment by an audiologist twice a year. They found that at routine ENT specialist

clinics, OM was detected in 55% of 184 examinations in Aboriginal children and the peak prevalence was 72% at age 5 to 9 months. TEOAEs were present in 90% (46/51) of Aboriginal children aged less than one month and in 62% (21/34) children aged 1 to 2 months. The children who failed TEOAEs at age 1 to 2 months were 2.6 times more likely to develop OM subsequently than those who passed. In view of this high prevalence of middle ear pathology in the Aboriginal infants, it is important to document the middle ear function at birth for this population.

1.6.2 Caucasian infants versus Aboriginal infants

When compared to Australian Aboriginal infants, Caucasian infants have a lower prevalence of ear disease. McGilchrist and Hills (1986) estimated that by 14 years, an Aboriginal child is likely to have spent two of those years with ear infections, compared to two months for his/her Caucasian counterpart. Moran, Waterford, Hollows and Jones (1979) found that only 1.3% of the 15,540 Caucasian children had OM in one or both ears compared to 16.5% of 21,988 Aboriginal children. Boswell and Nienhuys (1995) reported that compared to 95% of 22 six-to-eight-week old Aboriginal infants with MEE, only 30% of 10 Caucasian infants had MEE. Lehmann et al. (2008) found that 26% of 180 Caucasian children had OM during routine ENT examination between birth and 2 years of age. Peak prevalence was 40% at 10 to 14 months. TEOAE responses were present in 99% (120/121) of Caucasian children aged less than one month and in 93% (108/116) of children at 1 to 2 months of age. Leach et al. (1994) attributed the difference in ear disease in Aboriginal and Caucasian infants to differences in bacterial colonisation. They found that Caucasian infants had colonisation by only one species at a rate of 1% per day in comparison with Aboriginal infants who had colonisation with multiple species of respiratory bacteria at a rate of 5% per day.

Except for studies by Boswell, Nienhuys, Rickards, and Mathews (1993), Boswell and Nienhuys (1995) and Lehmann et al. (2008), there have been no other studies that have compared middle ear function at birth between these populations. Further, the existing studies have investigated middle ear function in Aboriginal neonates and young infants using otoscopy, 226 Hz tympanometry or TEOAE. It is well known that otoscopy and 226 Hz tympanometry are not reliable measures of middle ear function in young infants. Driscoll, Kei and McPherson (2000) suggested

that TEOAE alone is not sensitive to minor middle ear dysfunction and TEOAEs should be used along with another measure of middle ear function such as tympanometry.

Further research is needed to compare the outer and middle ear status at birth between Aboriginal and Caucasian neonates using appropriate methods such as 1000 Hz tympanometry and other middle ear measures. This comparison would provide evidence of any differences in the outer and middle ear function between the two groups at birth. This information would be useful for designing appropriate screening and intervention programs in relation to early detection, audiological and medical management, community health promotion and hearing loss prevention.

1.7 Non-Audiological Diagnosis of Middle Ear Effusion in Neonates and Infants

There are many ways to diagnose MEE in humans. Non-audiologic methods include otoscopy, pneumatic otoscopy, otomicroscopy and myringotomy. The accuracy in detecting MEE depends on the method, the client and the tester's skills and experience in performing the procedure.

1.7.1 Otoscopy.

Otoscopy is the most common clinical tool used to identify MEE in older children and adults. However, the accuracy of otoscopy in identifying MEE in young infants has been questioned as changes in colour, reflexive reaction to light, translucency and mobility of the eardrum have been found in healthy full term infants from birth to beyond 4 months of age (Cavanaugh, 1987; Jaffe, Hurtado & Hurtado, 1970; McLennan & Webb, 1957). Anatomical differences in the tympanic membranes and external auditory meati of neonates compared to older infants have been suggested to contribute to these otoscopic changes (Jaffe et al., 1970; McLellan & Webb, 1957). Anatomical development in early infancy include changes in the orientation and flexibility of the tympanic membrane and ossicular chain, rapid increase in the ear canal diameter and length (Keefe et al., 1993), formation of bony floor by 12 months (Kenna, 1990), and the middle ear cavity reaching adult size by six months of age (Eby & Nadol, 1986).

Even experienced physicians have found otoscopy in neonates to be onerous due to difficulties in visualising the tympanic membrane (Doyle et al., 1997; Zarnoch and Balkany, 1978). When successfully viewed, interpretation has been problematic due to less distinct landmarks (McLennan & Webb, 1957). Furthermore, inter-observer agreement regarding the presence of MEE in neonates has been reported to vary from 27% (laRossa, Mitchell, & Cardinal, 1993) to 85% (Marchant et al., 1986). Due to this large variation, Roberts et al. (1995) concluded that otoscopy could not be relied upon in this age group.

1.7.2 Pneumatic otoscopy.

Although pneumatic otoscopy has often been used with children and adults to identify MEE (Finitzo, Friel-Patti, Chinn, & Orval, 1992; Toner & Mains, 1990; Vaughan-Jones & Mills, 1992), it has not been successfully utilised with young infants.

Cavanaugh (1987) performed pneumatic otoscopy on 81 healthy full term babies during the first 72 hours of life and at routine well baby follow up visits. He found that limited mobility, changes in colour, poor lustre and relative opacity of the tympanic membrane occur in healthy neonates and may reflect the physiologic changes unique to the neonate period. Cavanaugh found that only 14 of 115 (12%) of the eardrums were visualised during the first three days of life. The success rate increased to 29 of 65 (44%) and 50 of 71 (71%) by 3 weeks and 10 weeks of age, respectively. In conclusion, Cavanaugh remarked that dullness of the tympanic membrane, decreased light reflex and diminished translucence occurred in greater than 90% of the neonates during the first three days of life. These occurrences declined to 26% or less by four months of age.

Marchant et al. (1984) reported that in infants, especially preterm infants, the ear canals are narrow and their tympanic membranes are less compliant to pneumatic insufflation than are those of older infants and children. Cavanaugh (1989) recorded pneumatic otoscopy in 53 healthy paediatric patients and found that there was a wide variation in pressures generated with the pneumatic otoscope. Hence, Cavanaugh suggested exploring the feasibility of modifying the instrument into a standardized objective system. In conclusion, due to a combination of physiological development

and difficulty in interpretation, pneumatic otoscopy has limited use with young infants.

1.7.3. Otomicroscopy.

Otoscopy using a binocular microscope, referred to as otomicroscopy, is reported to be superior to other methods of otoscopy because of significant improvement in sight due to magnification and three dimensional vision offering depth perception (McHugh & Traynor, 2009).

Young, TenCate, Ahmad, and Morton (2009) compared the findings of otomicroscopy by two specialist otolaryngologists with myringotomy results and found that otomicroscopy had overall accuracy of 94.1% with a sensitivity of 94.4% and specificity of 93.8%. The authors concluded that otomicroscopy performed by a specialist otolaryngologist is an accurate tool for the diagnosis of MEE. Lee and Yeo (2004) compared the findings of otomicroscopy, pneumatic otoscopy and tympanometry with myringotomy outcomes and found that otomicroscopy was the most sensitive (sensitivity of 100%) and specific (specificity of 61.5%) of the three diagnostic tests. However, despite high accuracy of otomicroscopy in the identification of MEE, its use with young infants has been limited as it needs to be done by a specialist and often requires anaesthetisation which is not justified in young infants with suspected MEE.

1.7.4 Myringotomy.

Myringotomy and aspiration of middle ear fluid under general anaesthesia is the current 'gold standard' for diagnosis of MEE (Maw, 1995). Myringotomy is a surgical procedure that involves making a tiny incision on the ear drum to relieve pressure caused by the build up of fluid in the middle ear. However, the main limitation of myringotomy is that it can only be justified in patients with specific indications, such as prolonged MEEs or recurrent OM. In young infants, wherein the findings can be influenced by normal age-related physiological changes, the use of myringotomy in research involving healthy cases is neither ethical nor justified.

1.8 Audiological Diagnosis of MEE in Neonates and Young Infants

Since MEE and associated conductive loss have been reported to cause increased referrals from newborn hearing screening, audiologists have become involved with the identification of middle ear pathology in infants in an attempt to differentiate sensorineural from conductive conditions. A variety of objective audiological tests have been utilised in the assessment of the outer and middle ear functions in neonates and young infants.

1.8.1 Tympanometry using low frequency probe tones (220/226 Hz).

Although tympanometry using a low frequency probe tone of 220/226 Hz has been accepted as the standard and objective clinical test for evaluation of middle ear problems (except for ossicular chain fixation) in older children and adults, its application has been limited in young infants (Hunter & Margolis, 1992; Paradise, Smith, & Bluestone, 1976; Shahnaz et al., 2009). Traditional 226 Hz tympanometry has been reported to produce incorrect test outcomes in young infants because normal results have been obtained in the presence of confirmed middle ear diseases in infants less than six months of age (Balkany et al., 1978; Beery, Andrus, Bluestone, & Cantekin, 1975; Hunter & Margolis, 1992; Keefe et al., 1993; Keefe & Levi, 1996; McKinley, Grose, & Roush, 1997; Meyer, Jardine, & Deverson, 1997; Paradise et al., 1976; Rhodes, Margolis, Hirsch, & Napp, 1999; Shurin, Pelton & Klein, 1976; Weatherby & Bennett, 1980; Williams et al., 1995; Zarnoch & Balkany, 1978). Paradise et al. (1976) found that about 40% of infants aged less than six months with confirmed MEE via pneumatic otoscopy and myringotomy findings exhibited normal, single peaked (Type A) tympanograms based on the Liden/Jerger classification system (Jerger, 1970; Liden, 1969). Other studies have found type A tympanograms in 20 to 94% of infants with confirmed MEE (Pestalozza & Cusmano, 1980; Schwartz & Schwartz, 1980).

Investigators have also found that low-frequency (e.g., 226 Hz) probe tone tympanometry can produce Type B tympanograms (i.e., no change in static compliance with pressure variation) in normal middle ears of young infants (Keefe et al., 1993; Keefe & Levi, 1996). Groothuis, Sell and Wright (1978) found that 7% of 71 infants aged 4 weeks to 17 months with normal otoscopic findings had type B tympanograms. Wright, McConnel, Thompson, Vaugh and Sell (1985) found that

37% of infants aged less than 6 months with normal pneumatic otoscopy results had type B tympanograms.

Furthermore, a high proportion of complex multi-peaked tympanograms have been obtained in infants compared with adults and older children with low frequency probe tones (Holte, Margolis & Cavanaugh, 1991; Keith, 1973; Sprague, Wiley, & Goldstein, 1985). These complex multi-peaked tympanograms did not fit to any category in either the Liden/Jerger scheme (Jerger, 1970; Liden, 1969) or the Vanhuyse model (Vanhuyse, Creten, & van Camp, 1975), thereby rendering the interpretation of results difficult and susceptible to errors.

Several studies have suggested that developmental changes in the outer and middle ear of infants and their physical properties in the first few months of life contribute to the differences in the tympanometry patterns seen with infants (Himelfarb, Popelka & Shanon, 1979; Holte et al., 1991; Hunter & Margolis, 1992; Keefe et al., 1993; McKinley et al., 1997; Meyer et al., 1997). Some of the developmental changes in the infant ear include an overall increase in the size of the ear canal and middle ear space, decrease in the length of the cartilaginous portion of the ear canal due to growth of the bony portion of the canal wall, decrease in the overall mass of the middle ear due to loss of residual mesenchyme and changes in the ossicular bone density and ossification, changes in the orientation and flexibility of the tympanic membrane and ossicular chain, ossicular joint tightening, and a lesser coupling between the stapes and the annular ligament (Ikui, Sando, Sudo, & Fujita, 1997; Ruah, Schachern, Zeltermann, Paperella & Yoon, 1991; Saunders, Kaltenback, & Relkin, 1983).

Cumulatively, these developmental changes result in a mass-governed middle ear transmission system which gradually transforms into an adult-like stiffness-dominated system (Himelfarb et al., 1979; Holte et al., 1991; Hunter & Margolis, 1992; McKinley et al., 1997; Meyer et al., 1997; Saunders et al., 1983). As the infant ear changes from a mass-dominated to a stiffness-dominated system, the resonance frequency of the outer and middle ear system increases. Meyer et al. (1997) found the resonance frequency to be below 550 Hz in an infant till she was 14 weeks old. By three to four months, the resonance frequency reached 800 to 1200 Hz similar to that

of adults. For this reason, they suggested that low frequency tympanometry that is used to evaluate a stiffness based middle ear system was not appropriate for the mass governed middle ear of the young infant. Other studies too have supported this view and suggest that due to these maturational changes, tympanometry using 226 Hz is invalid in infants below six months of age (Holte et al., 1991; Keefe & Levi, 1996).

1.8.2 Tympanometry using high frequency probe tones (600/678 and 1000 Hz).

Due to the limitation of 226 Hz probe tone tympanometry in young infants, the use of high frequency probe tone has been recommended for this population. Investigators have used probe tones of 660/678 Hz and 1000 Hz in evaluating the infant middle ear (Alaerts, Luts & Wouters, 2007; Baldwin, 2006; Beery et al., 1975; Calandruccio et al., 2006; Harris, Hutchinson, & Moravec, 2005; Kei et al., 2003; Marchant et al., 1986; Margolis et al., 2003; Purdy & Williams, 2002; Swanepoel et al., 2007).

1.8.2.1 Tympanometry using a 660/678 Hz probe tone

Several studies have shown that 660/678 Hz probe tones are more accurate in diagnosing MEE than the traditional 220 Hz probe tone in infants (Himelfarb et al., 1979; Marchant et al., 1986; Shurin et al., 1976; Sprague et al., 1985; Sutton, Gleadle, & Rowe, 1996). In 1986, Marchant and colleagues demonstrated good agreement between otoscopy and 660 Hz probe tone tympanometry (Kappa coefficient = 0.86) in 86 infants less than 5 months of age. Shurin et al. (1976) found that 660 Hz tympanometry provided better separation between normal ears and ears with MEE than 220 Hz tympanometry. Based on a combination of pattern classification and susceptance criteria in 91 children, Beery et al. (1975) found oto-admittance at 660 Hz to be a better indicator of effusion than at 220 Hz. They found that 660 Hz tympanometry performed just before myringotomy accurately predicted MEE in 96% of ears when effusion was present and in 93% of ears predicted no effusion when the ears were actually dry.

However, the clinical utility of 660/678 Hz tympanometry has been questioned due to the presence of complex multi-peaked tympanograms with this probe tone. McKinley et al. (1997) used a 678 Hz probe tone in evaluating the middle ear function

of 55 healthy neonates and found that 18% of the multi-peaked tympanograms were classified as unusual or “other”. In another study, Himelfarb et al. (1979) found that 85% of the tympanograms recorded were multi-peaked. They attributed this phenomenon to the high compliance of infants’ external ear canal walls. This finding is substantiated by Keefe et al. (1993) who measured acoustic impedance, admittance and reflection from 125 to 10,700 Hz and found the transmission of sounds between 220 and 660 Hz into the middle ear was not efficient due to ear canal wall vibration and resonance. Hence, Keefe et al. concluded that 220 to 660 Hz is a poor frequency range to use for tympanometry with infants. They recommended that frequencies between 1000 and 4000 Hz should be used for testing infants because this sound can be most efficiently transmitted into the middle ear.

1.8.2.2 Tympanometry using a 1000 Hz probe tone

Tympanometry utilising a probe tone of 1000 Hz can produce more reliable and accurate results than 226 Hz or 600/678 Hz tympanometry in detecting MEE in young infants (Baldwin, 2006; Kei et al., 2003; Margolis et al., 2003; Mazlan et al., 2007; Meyer et al., 1997; Purdy & Williams, 2002; Rhodes et al., 1999; Williams, Purdy & Barber, 1995). Williams et al. (1995) studied 26 infants under four months of age and found that the peak susceptance at 1000 Hz provided the best agreement with otomicroscopy and pneumatic otoscopy. In their study of 87 NICU babies, Rhodes et al. (1999) found that 30 to 67% of babies who failed the 226 Hz and 678 Hz tympanometry actually passed a series of electrophysiological tests including OAE and ABR. In contrast, they found that the three ears that failed 1000 Hz tympanometry also failed the OAE and ABR tests. In a longitudinal study of a child from two weeks to 6.5 months of age, Meyer et al. (1997) utilised both 226 and 1000 Hz probe tones and found that 1000 Hz tympanometry provided better diagnostic sensitivity to middle ear dysfunction than conventional 226 Hz tympanometry.

However, routine adoption of 1000 Hz tympanometry for neonates has been hindered by difficulties surrounding trace interpretation. While interpretation of 226 Hz tympanograms obtained from adults has been well established (Beery et al., 1975; Jerger, 1970; Liden, 1969), there is no agreement on the interpretation of 1000 Hz tympanograms in infants. Several researchers have created their own methods to classify tympanometric findings in infants (Baldwin, 2006; Marchant et al., 1986;

Sutton et al., 1996; Williams et al., 1995). For example, Baldwin (2006) utilised the shape classification method initiated by Marchant et al. (1986) and proposed a classification system based on identifying positive versus negative peaks above a baseline between +200 and -400 daPa. Using this method of classification in the study of 211 young infants, Baldwin found that normal tympanograms were characterised by a positive peak while abnormal ones were of a negative or trough configuration. Using air and bone conduction ABR as the gold standard, Baldwin found the sensitivity and specificity of 1000 Hz tympanometry to be 0.99 and 0.89, respectively.

Other researchers have used simple visual classification systems in which the presence of a peak or notching is indicative of normal middle ear function and a flat or sloping tympanogram is suggestive of MEE. Using this method with 122 normal healthy neonates, Kei et al. (2003) found that 92.3% had single peaked (Type 1) tympanograms, 5.7% had flat (Type 2) tympanograms, 1.2% had double peaked (Type 3) tympanograms and 0.8% did not fit into any categories (others). The majority of infants had Type 1 tympanograms, similar to the Type A tympanograms of the conventional Liden/Jerger classification (Jerger, 1970; Liden, 1969) that are often seen in adults and children with normal middle ear function. Kei et al. (2003) concluded that the Type 1 tympanogram is indicative of normal middle ear function given the presence of normal TEOAEs, uneventful birth history and no predisposing high risk factors for hearing loss. This result corresponded well with the findings by Alaerts et al. (2007) who evaluated 110 children from birth to 32 months and found that 91% of infants younger than 3 months had Type 1 tympanograms. These findings are also consistent with that of Margolis et al. (2003) who found that the tympanograms obtained using a 1000 Hz probe tone in neonates were almost always single-peaked and free of artefacts and irregular patterns.

Swanepoel et al. (2007) classified 1000 Hz tympanograms from 278 ears of 143 healthy neonates aged one to four weeks and recorded only two types of tympanograms, single and double peaked patterns with a prevalence of 94% and 6%, respectively. However, in contrast to the findings of Kei et al. (2003), Swanepoel et al. (2007) suggested that double-peaked tympanograms were indicative of normal middle ear transmission because strong TEOAEs were obtained from these ears.

Several researchers have suggested the use of the Vanhuyse model (Vanhuyse et al., 1975) to interpret tympanometric results in infants and young children (Alaerts et al., 2007; Calandruccio et al., 2006; Holte et al., 1991; Sprague et al., 1985; Sutton et al., 1996). The Vanhuyse model defines four patterns of admittance tympanograms, based on the combined minima and maxima in both susceptance (B) and conductance (G) tympanograms, namely 1B1G, 3B1G, 3B3G and 5B3G. Alaerts et al. (2007) found the Vanhuyse model to be suitable for identifying 1000 Hz tympanograms in 110 children from birth to 32 months and in adults. An equal distribution of 1B1G and 3B1G types in the youngest children changed into a distribution with predominantly 3B1G types in adults. Calandruccio et al. (2006) found the Vanhuyse et al. model to be useful in classifying 1000 Hz tympanograms in young infants. They reported that the majority of infants (4 weeks to 6 months) had equal distribution between 1B1G and 3B1G tympanograms, while adults had predominantly 3B1G tympanograms. Similarly, both Alaerts et al. and Calandruccio et al. found an equal distribution of 1B1G and 3B1G types in the young infants while adults had predominantly 3B1G tympanograms.

Nevertheless, with the use of the Vanhuyse model, the interpretation of tympanometric patterns is more complicated compared with the simple visual admittance classification method. For example, McKinley et al. (1997) found that more than half of the 55 neonatal ears studied exhibited susceptance and conductance that could not be classified using the Vanhuyse multi-component tympanogram models. They found no clear relationship between the presence of TEOAEs and tympanograms, using 226, 678 and 1000 Hz probe tones, classified according to the Vanhuyse model of tympanometric shapes (Vanhuyse et al., 1975) and concluded that this model was not adequate for classifying and interpreting the majority of their high frequency neonatal tympanograms. Moreover, by merely looking at susceptance (B) and conductance (G) patterns, middle ear pressure and the extent of tympanic membrane movement are not taken into account. These constraints make the Vanhuyse model less applicable for middle ear assessment in neonates and young infants.

Alaerts et al. (2007) suggested the use of a combination of tympanogram shape and middle ear pressure for assessment of middle ear function in young infants.

They recommended pass criteria as presence of single peak and tympanic peak pressure around 0 daPa (Type 1 tympanogram) was equivalent to Type A of the Liden/Jerger classification system (Jerger, 1970; Liden, 1969). Tympanograms with a single peak and negative pressure of <-150 daPa were classified as Type 3 (or Type C in the Liden/Jerger classification system). More recently, attempts have been made to describe the characteristics of 1000 Hz tympanograms in neonates passing an OAE screen (Kei et al., 2003; Margolis et al., 2003). These studies have produced 5th and 95th percentile data for a variety of test parameters which, the authors believe, may serve as pass/fail criteria for 1000 Hz tympanometry (Kei et al., 2003; Margolis et al., 2003). Peak compensated static admittance (compensated at either the negative or positive pressure end) appears to be a common measure employed by researchers. For example, Mazlan et al. (2009) suggested using the 5th percentile (0.23 mmho) for positive tail (+200 daPa) peak compensated static admittance as a pass/fail criterion. However, this criterion was not evaluated with infants with abnormal middle ear function and, hence, the sensitivity and specificity of the test are not known. Margolis et al. (2003) recommended compensation performed at the negative pressure end (-400 daPa) and suggested a cut-off of at least 0.6 mmho as a pass criterion for infants up to four weeks of age. In a study of 278 neonatal ears, Swanepoel et al. (2007) found increased variability in the 95th percentile and suggested a 5th percentile cut off value at 1.4 mmho.

Mazlan et al. (2009) used a different approach with 1000 Hz tympanometry and compared two methods of obtaining middle ear admittances, namely, peak compensation and component compensation, in 42 neonates. They found that mean middle ear admittances obtained by compensating for the susceptance and conductance components at a pressure of +200 and -400 da Pa ($Y_{CC200} = 1.00$ mmho and $Y_{CC-400} = 1.24$ mmho, respectively) were significantly greater than those obtained using the traditional baseline compensation method ($Y_{BC} = 0.65$ mmho). Although Y_{CC-400} had the highest mean value, it had the lowest test-retest reliability. Therefore, they suggested that the component approach compensated at 200 daPa (Y_{CC200}) holds promise as an alternative method for estimating middle ear admittance in neonates.

From the above description of 1000 Hz tympanometry, it can be seen that there is no unanimous agreement on either the tympanometric shape classification or

the optimal test parameter for assessing middle ear function in infants. The meaningfulness of using conventional tympanometry, peak compensated admittance, tympanometric peak pressure, equivalent ear canal volume and tympanometric width to assess middle ear function in infants has not been clearly demonstrated. There are significant differences in the mean admittance proposed by various studies (Kei et al., 2003; Margolis et al., 2003; Swanepoel et al., 2007) which can be attributed to differences in using single versus both single- and double-peaked tympanograms, as well as differences in equipment, age of subjects and measurement techniques.

In general, research recommends that 1000 Hz tympanometry be used in the assessment of middle ear dysfunction in infants compared to 220 or 660 Hz tympanometry. However, further research is needed to develop standardised measures of the 1000 Hz tympanogram that can be universally accepted in the assessment and interpretation of test findings for this population. Until such measures are developed, 1000 Hz tympanometry should be used with caution along with other measures of middle ear function.

1.8.3 Acoustic stapedial reflex (ASR).

Although the ASR test has often been used for site of lesion testing to diagnose conductive, cochlear and retrocochlear pathologies in adults (Ferguson et al., 1996; Handler & Margolis, 1977; Jerger, Burney, Mauldin, & Crump, 1974), its application to young infants has been limited. Earlier studies using probe tone frequencies of 200 Hz have shown large variations in the prevalence of the ASR in neonates. Vincent and Gerber (1987) studied the ASR in neonates and six-week-old infants using activating stimuli (broadband noise and pure tones of 500, 1000, 2000 and 4000 Hz) and a probe tone of 220 Hz. They found that reflexes were present for all the five stimuli in 92.5% of 40 ears of neonates and 95% of 40 ears of six-week-old infants. In contrast, other studies that have used a 220 Hz probe tone to measure the ASR in neonates and young infants have observed absent reflexes in 90% of infants (Abahazi and Greenberg, 1977; Keith, 1973; Stream, Stream, Walker, & Brenningstall, 1978).

Other normative studies with neonates using 660/678 Hz probe tones have also demonstrated large variations in the prevalence of the ASR. Sprague et al. (1985)

utilised a probe tone of 660 Hz and found reflexes in 81% of 53 neonates. Sutton et al. (1996) used a probe tone of 678 Hz with neonates and found reflexes in 42% of 168 ears. In contrast, Keith (1973) found ASRs to 500 and 1000 Hz tones at 100 dB HL in only 6% of 40 healthy neonates aged 36 to 151 hours after birth, while Keith and Bench (1978) found ASRs in only 5.4% of infants.

Higher probe frequencies have been successfully employed in obtaining ASRs from neonates and young infants, with several studies demonstrating higher prevalence with higher frequencies. In a study utilising multiple probe tone frequencies from 220 to 2000 Hz, Weatherby and Bennett (1980) found that ASRs could be elicited in all 44 healthy neonates for a broadband noise activator when probe frequencies from 800 to 1800 Hz were used. Other studies that used a probe tone of 1000 Hz to elicit the reflex have also obtained similar findings (Mazlan et al., 2007, 2009; Rhodes et al., 1999; Swanepoel et al., 2007). For example, Rhodes et al. (1999) demonstrated ASRs in 87% of 173 babies in an NICU when a 1000 Hz probe tone and an activating stimulus of 2000 Hz was used. Bennett and Weatherby (1982) used a 1200 Hz probe tone to measure the ASR in neonates and were able to obtain the ASR in 26 out of 28 infants. Swanepoel et al. (2007) successfully reported ASRs in 94% of healthy young infants aged 1 to 28 days using a 1000 Hz probe tone and 1000 Hz activator. Using a 1000-Hz probe tone, Mazlan et al. (2007) demonstrated that ASRs could be recorded from all 42 healthy full term neonates when stimulated ipsilaterally by either a 2000 Hz pure tone or broadband noise stimulus.

Apart from the difference in the incidence of ASR with various probe tones, a large variation in the ASR threshold with probe tone frequencies has also been reported. For example, the mean acoustic reflex threshold has been shown to be 11 dB higher with a 1000 Hz probe tone compared to other probe tones. Swanepoel et al. (2007) obtained a reflex threshold of 93 dB using a 1000 Hz probe tone and 1000Hz ipsilateral stimulus for neonates. This threshold was higher compared to the mean threshold of 82 dB using a 660 Hz probe tone to elicit ipsilateral ASRs in neonates (Sprague et al., 1985) and 82 dB using a 220 Hz probe tone in adults (Wiley, Oviat, & Block, 1987). Hirsch, Margolis, and Rykken (1992) utilised a probe tone of 800 Hz with high risk infants and found that the mean ASR threshold was approximately 15 dB lower for the broadband noise compared to pure tone stimulus. This finding is

consistent with that obtained by Mazlan et al. (2007), who found a 13.7 dB difference in ipsilateral ASR thresholds between the 2000 Hz and broadband noise stimuli while testing 42 healthy neonates. In another study of 194 neonates, Mazlan (2009) found a 11.3 dB difference between 2000 Hz and broadband noise using a 1000 Hz probe tone.

In addition to the increased variation in the frequency and threshold of ASRs in infants, earlier studies have shown contrasting findings regarding the clinical utility of the ASR in the middle ear assessment of infants. For example, Hirsch et al. (1992) used ASR in conjunction with ABR for screening 76 babies from an NICU and found that 12 ears with elevated or absent reflexes also had delayed ABR wave latencies and concluded that combined information from ABR and ASR might be valuable for early detection of MEE in infants. In another study, Plinkert, Sesterhenn, Arold, and Zenner (1990) used ABR, ASR and TEOAE to screen 53 infants and found that ASR correctly predicted normal hearing in 78% of ears that had normal ABR thresholds (<30 dB nHL), compared with 91% for TEOAEs. They proposed that the ASR-TEOAE combination could be an efficient screening tool.

Marchant et al. (1986) measured ipsilateral acoustic reflex thresholds using a 660 Hz probe tone in 86 infants below five months of age, and found that optimal agreement was obtained between otoscopically normal ears and those with middle ear effusion using a threshold of >100 dB HL as the criterion for MEE. However, improved agreement could not be achieved by adding reflex thresholds to peak susceptance. Therefore, they recommended that either peak susceptance or ipsilateral ASR could be used for the detection of MEE in early infancy.

Despite conflicting reports on the clinical utility of ASRs in neonates and young infants, ASR testing with a probe tone of 1000 Hz has been found to give optimal results. Apart from the studies by Mazlan et al. (2007, 2009), there are very few normative studies on ipsilateral ASR using 1000 Hz tone in infants. Hunter et al. (2013), however, reported that standard acoustic reflex tests may have a risk of iatrogenic hearing loss due to the need for high stimulus levels. More research on the application of ASR with infants is required before it can be used routinely in paediatric audiology clinics.

1.8.4 Transient evoked otoacoustic emissions (TEOAE) and Distortion Product otoacoustic emissions (DPOAE).

The presence of OAEs has often been used as an indicator of normal middle ear function, especially in infants, in lieu of procedures like pneumatic otoscopy or myringotomy. There are two main types of evoked OAEs, namely TEOAE and DPOAE. Studies utilising TEOAE as an indicator of normal middle ear function have utilised the frequency range of 1500 to 4000 Hz (Kei et al., 2003; Margolis et al., 2003; Swanepoel et al., 2007). Studies utilising DPOAE as an indicator of normal middle ear function have utilised the frequency range of 2000 to 6000 Hz (Merchant et al., 2010; Sanford et al., 2009; Swanepoel et al., 2007; Vander Werff et al., 2007).

As OAEs require efficient transmission of sound from the outer to the inner ear and vice versa, normal OAE results provide some level of assurance of normal outer and middle ear function (Hunter et al., 2010; Kei et al., 2003; Margolis et al., 2003; Sanford et al., 2009; Shahnaz, 2008). OAEs are affected by even slight changes in the condition of the outer and middle ear (Prieve & Dreisbach, 2011). For instance, TEOAE and DPOAE levels are reduced in infants and children having negative tympanometric peak pressure (TPP) (Choi, Pafitis, & Zalzal, 1999; Hof, Anteunis, Chenault, & Van Dijk, 2005; Hof, Van Dijk, Chenault, & Anteunis, 2005; Koike & Wetmore, 1999; Koivunen, Uhari, Laitakari, Alho, & Luotonen, 2000; Lonsbury-Martin, Martin, McCoy, & Whitehead, 1994; Owens, McCoy, Lonsbury-Martin, & Martin, 1992; Prieve, Calandruccio, Fitzgerald, Georgantas, & Mazevski, 2008). Prieve and colleagues (2008) measured TEOAEs in infants when their TPP was normal and again when it was negative. They found an approximately 4 dB reduction in TEOAE response level for the frequency bands from 1000 to 4000 Hz, while the mean change in TPP between the two measurements was -169 daPa. In comparison, children with flat tympanograms show dramatically reduced OAE level or no measurable OAEs (Choi et al., 1999; Koike & Wetmore, 1999; Koivunen et al., 2000; Lonsbury-Martin et al., 1994). Absent TEOAEs has been reported to be a common finding in children with confirmed OM, most often when middle ear fluid is viscous (Amedee, 1995) or when there is a large quantity of effusion (Koivunen et al., 2000).

However, passing the TEOAE or DPOAE, in its strictest sense, cannot serve as a “gold-standard” for normal middle ear function because both TEOAE and DPOAE have been found to be present in some ears with middle ear dysfunction in children and adults (Driscoll et al., 2000; Kemp, 2002; Sanford et al., 2009; Taylor & Brooks, 2000; Thornton et al., 1993; Van Cauwenberge, Vinck, De Vel and Dhooge, 1995). Despite this limitation, the OAE test has been widely used to assess the integrity of the conductive pathway (outer and middle ear) in neonates and young infants without resorting to invasive procedures, such as myringotomy, that carry risk and are not ethical in otherwise healthy neonates. Presently, TEOAEs/DPOAEs serve as a surrogate gold standard for evaluating the test performance of other measures in identifying OM in young infants.

1.8.5 Auditory brainstem response (ABR).

ABR is currently considered as the gold standard measure for assessing the auditory function of infants referred from NHS. ABR thresholds can be measured using either clicks or tone bursts as stimuli. ABR using click stimuli provides a global measure of physiological thresholds. In contrast, ABR using tone burst stimuli provides frequency specific threshold information. Normal hearing infants show mean thresholds of about 15 to 20 dB nHL from 500 to 4000 Hz (Lee, Hsieh, Pan, & Hsu, 2007; Rance, Tomlin, & Rickards, 2006; Stapells, 2000, 2011; Vander Werff, Prieve, & Georgantas, 2009).

The most common cause of elevated ABR thresholds in young infants, especially those referred from NHS, is conductive hearing loss (Boone et al., 2005; Gravel, 2002). Immittance and OAE measures are unable to quantify the degree of conductive hearing loss. In the presence of conductive pathology, these measures are typically abnormal irrespective of whether the conductive component is relatively minor or substantial (Stapells, 2011). Analysis of ABR wave V or wave I latencies in response to air conducted clicks is reported to differentiate conductive from sensorineural losses and even quantify the conductive component (Fria & Sabo, 1979; McGee & Clemis, 1982; Yamada, Yagi, Yamane, & Suzuki, 1975). However, attempts to quantify the amount of conductive component using air conduction click ABR wave V or wave I latency shifts have not proven to be reliable with large errors in many infants (Eggermont, 1982; Mackersie & Stapells, 1994) and relatively low

correlations between latency and the size of the air-bone gap (Vander Werff et al., 2009). For example, Stapells (2011) reported differences in ABR patterns in an infant with bilateral OM. Bone conduction thresholds were normal. Wave V thresholds to air conducted stimuli were normal (20 dB nHL or better) for the left ear, but mildly elevated (40 dB nHL) for the right ear. Despite the difference in thresholds, wave V latencies were well within normal limits in both ears. It is, therefore, not possible to reliably determine the presence or degree of a conductive component using air conduction ABR latency information.

While air and bone conduction (AC and BC) tone burst ABR may be regarded as a surrogate gold standard for detecting conductive conditions, this threshold ABR measure requires long testing time to complete and is done as a diagnostic measure at some point later in time rather than during the hearing screening period. This measure is not sensitive to detecting subtle conductive conditions where the AC tone burst ABR thresholds are within normal limits. Despite the practice of BC tone burst ABR being in place for over two decades (Gravel, Kurtzberg, Stapells, Vaughan, & Wallace, 1989; Stapells & Ruben, 1989), Stapells (2011) reported that many clinicians do not routinely obtain ABR results for BC stimuli after finding elevated AC thresholds. Instead, they rely on immittance results for determining the middle ear status. Stapells attributed this to several reasons including limited dynamic range, the use of 10-dB step size and lack of published data for AC-BC differences in infants. Stapells also reported that when BC testing is performed, the results are not used to calculate the air-bone gap but are primarily used to indicate whether bone thresholds are normal or elevated.

1.8.6 Auditory steady state response (ASSR).

The ASSR responses to stimuli presented using repetition (or modulation) rates in the 70 to 110 Hz range have recently gained considerable attention in the clinical evaluation of infants. An ASSR is a repetitive evoked potential, which is best considered in terms of its constituent frequency components rather than in terms of its waveform (Regan, 1989). The primary goal of ASSR audiometry is to estimate behavioural thresholds with application of regression formula or correction factors (Rance et al., 2005; Stapells, Gravel, & Martin, 1995).

The normative ASSR database for infants has only been developed in recent years (Han, Mo, Liu, Chen, & Huang, 2006; Luts, Desloovere, & Wouters, 2006; Rance & Briggs, 2002) and is less than well understood. Its application in the evaluation of conductive hearing loss has been limited as there is no agreement across the studies due to differences in the stimuli (single or multiple frequencies) or analysis techniques (signal-to-noise ratio or noise criteria) used (Stapells, 2011).

Assessment of conductive hearing loss in infants and children using ASSR has received very little attention. There appears to be only one study of AC ASSR thresholds in children with various types of hearing loss including conductive hearing loss (Swanepoel, Ebrahim, Friedland, Swanepoel, & Pottas, 2008). Despite the promising results in Swanepoel et al.'s (2008) study, Stapells (2011) pointed out that the degree of conductive loss could not be estimated based on the frequency-specific AC and BC thresholds. Instead AC click ABR, TEOAE, tympanograms and otoscopy, none of which can provide an estimate of the size of the conductive component, were used.

ASSRs to BC stimuli have not been thoroughly investigated. BC ASSRs have been reported on normal and premature infants (Hulecki & Small, 2011; Small & Hansen, 2012; Small & Stapells, 2008; Stapells, 2011). But, currently there are no published BC ASSR studies in infants with middle ear pathology and hearing loss. Further research is required especially in infants with confirmed middle ear pathology and hearing loss confirmed by behavioural tests or tone burst ABRs to AC and BC stimuli.

It can be seen from the above studies that there is no single clinical measure that can be effectively utilised to measure middle ear function in neonates and young infants. The available audiological and non-audiological measures do have limitations. Assessing conductive disorders in young infants is a real challenge due to the lack of effective and objective tools for detecting these disorders for this population (Kei and Zhao, 2012). Paediatric audiologists, therefore, need sensitive, specific and user friendly screening and diagnostic tool that permit accurate determination of outer and middle ear status (Hunter et al., 2013).

1.9 Wideband Acoustic Immittance (WAI)

Wideband acoustic immittance (WAI) refers to a family of wideband measures including power absorption, transmittance or power absorption in decibels, acoustic impedance (resistance and reactance) and admittance (conductance and susceptance). These physiological measures evaluate outer and middle ear function independently of the inner ear. They provide detailed information about acoustic-mechanical properties of the outer and middle ear across the frequency range most important for speech perception (Keefe, 2008; Keefe et al., 1993; Keefe & Levi, 1996). Currently, the most commonly used measures are wideband reflectance and absorbance. WAI measures can be performed under ambient pressure or pressurised conditions.

1.9.1 WAI under ambient pressure conditions.

1.9.1.1 *Wideband reflectance*

Wideband reflectance (WBR) is the most frequently used measure of WAI. It is also known as power reflectance, energy reflectance or reflectance. WBR is the ratio of reflected power to incident power. It ranges from 0 (representing complete transfer of sound into the middle ear) to 1 (representing no sound transferred to the middle ear) (Voss & Allen, 1994). WBR is the square of the pressure reflectance. Mathematically, WBR is a real number expressed only in magnitude and not phase.

1.9.1.2 *Wideband absorbance*

Wideband absorbance (WBA), also known as power absorbance, energy absorbance or absorbance, is the complement of WBR and expressed as $WBA = 1 - WBR$ (Sanford et al., 2013; Neely, Stenfalt, & Schairer, 2013). Similar to WBR, WBA is also a real number without a phase component. WBA is defined as the ratio of energy absorbed by the middle ear to the incident energy and varies from 0 (no energy transferred to middle ear) to 1 (complete transfer of energy into middle ear) (Feeney & Sanford, 2012). In the present study, WBA rather than WBR was measured in neonates and young infants as recommended by Feeney et al. (2013). The terms WBA and absorbance are used interchangeably in this thesis. To ensure consistency and ease of understanding of earlier studies, the findings of these studies are discussed in terms of the WBA measure, although WBR was actually measured in these studies.

Several normative studies have shown that, at all ages, power absorbance is lowest at frequencies below 1000 Hz and above 4000 Hz and highest in the frequency region between 1000 and 4000 Hz, which corresponds to the most effective frequency region of the middle ear transfer function (Feeney et al., 2003; Keefe et al., 1993; Hunter, Tubaug, Jackson & Propes, 2008b; Margolis, Saly & Keefe, 1999; Sanford & Feeney, 2008; Sanford et al., 2009; Voss & Allen, 1994).

According to Keefe et al. (2000), WBA has several advantages over tympanometry, OAE and ABR: WBA is a very fast test, requiring only several seconds to acquire a response; unlike tympanometry, it can measure energy transmission under ambient pressure conditions; it measures a wide range of frequencies from 250 to 8000 Hz and provides more detailed information on the status of the outer and middle ear; it provides clinical information on the range of frequencies crucial for speech perception, and it is less susceptible than OAE and ABR measurements to environmental and subject noise.

1.9.1.3 WBA in adults.

Earlier reports on WBA described the absorbance pattern across the frequencies in adults. Margolis et al. (1999) studied 20 adults aged 20 to 53 years and found that the absorbance pattern was characterized by low absorbance at low frequencies, two distinct maxima at approximately 1200 and 3500 Hz, and decreasing absorbance below 1200 and above 3500 Hz. Other studies have reported increased absorbance between 1000 and 4000 Hz (Keefe et al., 1993; Sanford and Feeney, 2008; Shaw & Stinson, 1981; Voss & Allen, 1994). Figure 1.1 illustrates this WBA pattern in normal adults across two studies (Keefe et al., 1993; Sanford & Feeney, 2008). On the other hand, Zhao, Lowe, Meredith, and Rhodes (2008) studied WBA in 50 normal ears of 25 adults and described three types of WBA configurations: (i) Type I with symmetric 'M' shape wherein two peaks were present in the low to mid and high frequency bands with central frequency of each peak around 1000 Hz and 4000 Hz, respectively; (ii) Type II with asymmetric 'M' shape wherein two peaks were present with the central frequency of the peak in the low to mid frequency band being shallower than that of the high frequency band, and; (iii) Type III with 'inverted U' shape with a single rounded peak with the central frequency between

2000 and 5000 Hz. Zhao et al. (2008) attributed the peaks to the resonances in the outer and middle ear.

It can be seen that normative WBA patterns obtained in adults across the studies have not been consistent. Further studies are necessary to develop normative WBA data in adults. Such studies are necessary in order to analyse differences in WBA across age, gender and ethnicity (Shahnaz, Feeney & Schairer, 2013).

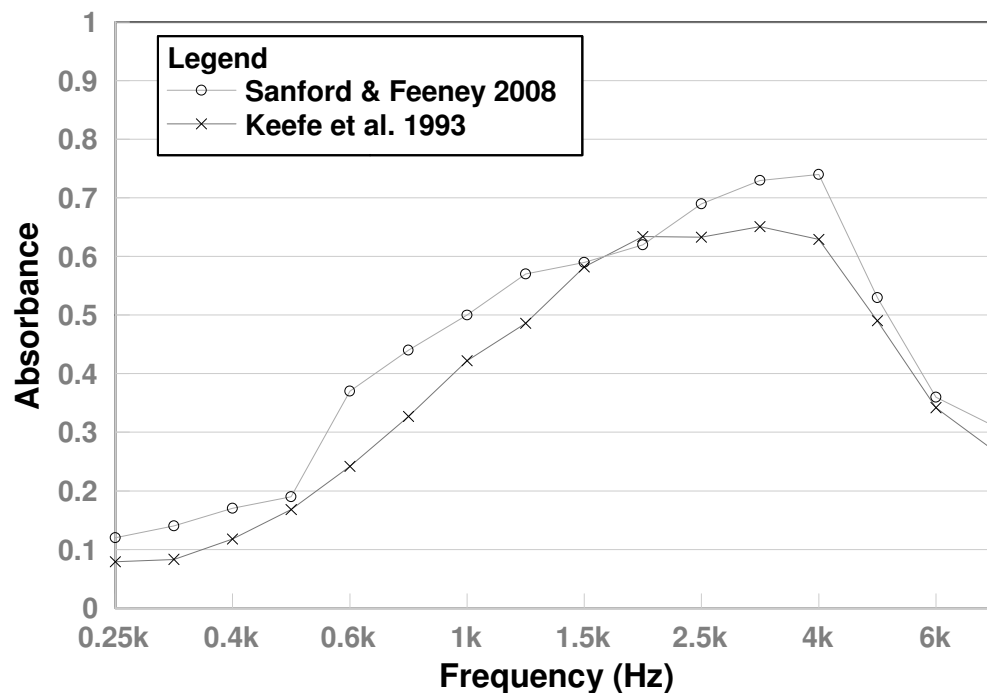


Figure 1.1: Wideband absorbance in adults across two studies.

1.9.1.4 WBA in children.

There are very few studies investigating WBA in children. Beers et al. (2010) established normative WBA data for 78 children with an average age of 6.15 years. Several studies have measured WBA in children with normal auditory function with the purpose of comparing results with those obtained from children with middle ear dysfunction (Beers et al., 2010; Jeng et al., 1999; Keefe & Simmons, 2003; Margolis, Saly & Hunter, 2000). Jeng et al. (1999) obtained WBA measurements on 30 normal ears from 15 children in the age of range of two and a half to five years and found the associated absorbance pattern to be similar to that obtained from normal adults by

other researchers (Keefe and Bulen, 1992; Keefe et al., 1993; Voss & Allen, 1994). Jeng et al. reported that relatively little power was absorbed by the ear at low frequencies with the amount of power absorption increasing steadily with increasing frequency. At 1000 Hz, just over half of the acoustic power entering the ear canal was absorbed by the middle ear and cochlea. Peak power absorption was reached in the 3000 to 5000 Hz region for most of the subjects and almost all of the acoustic power was absorbed at these peak frequencies. Power absorption then rapidly decreased at higher frequencies above 5000 Hz. Margolis et al. (2000) also obtained similar results in a WBA study of 12 normal ears from eight subjects aged nine to 16 years.

Keefe and Simmons (2003) studied 42 normal functioning ears in adults and children aged 10 years and up and found absorbance of approximately 0.2 near 250 Hz, increasing with increasing frequency to a maximum of approximately 0.9 in the frequency range of 2500 to 3000 Hz and decreasing to lower values at 8000 Hz.

From this review of WBA studies in children, it can be seen that there is a lack of large scale, normative reports in this population. In particular, the age range of children in the available WBA studies varied greatly. Further studies are needed to obtain age-specific normative data for children, which may show changes in WBA with age.

1.9.1.5 WBA in neonates

WBA has been successfully measured in healthy neonates as well as neonates in NICU. A summary of studies that have investigated WBA under ambient pressure conditions in neonates is provided in Table 1.1. The general pattern of WBA in neonates is reported to be similar to that seen in children and adults with absorbance being highest between 1000 and 4000 Hz and decreasing below 1000 and above 4000 Hz. Figure 1.2 illustrates WBA measured in neonates across five studies.

Keefe et al. (2000) conducted the first study of WBA in 2081 neonates. The participants were divided into three groups: neonates in NICU, neonates in the well baby nursery without any risk factors for hearing loss and neonates in the well baby nursery with one or more risk factors associated with hearing loss. The investigators found a median absorbance of about 0.8 across all frequencies from 250 to 8000 Hz.

They also found significant ear and gender effects on absorbance in some frequency bands. In particular, the Keefe et al data showed greater WBA at low frequencies than those of other studies. Contributing factors include evidence of inadequate probe seal during testing in 13% of neonates and that the neonates were not screened for any conductive conditions before participating in the study.

In a study of WBA in NICU infants, Shahnaz (2008) investigated 26 neonates with a mean gestational age of 37.8 weeks and compared the results to WBA measures obtained from normal hearing adults. Shahnaz found a clear separation between NICU babies and adults below 727 Hz, with NICU babies having higher absorbance values than adults. The NICU neonate mean absorbance was larger at all frequencies than the corresponding mean for one-month-old infants from Keefe and Levi (1996).

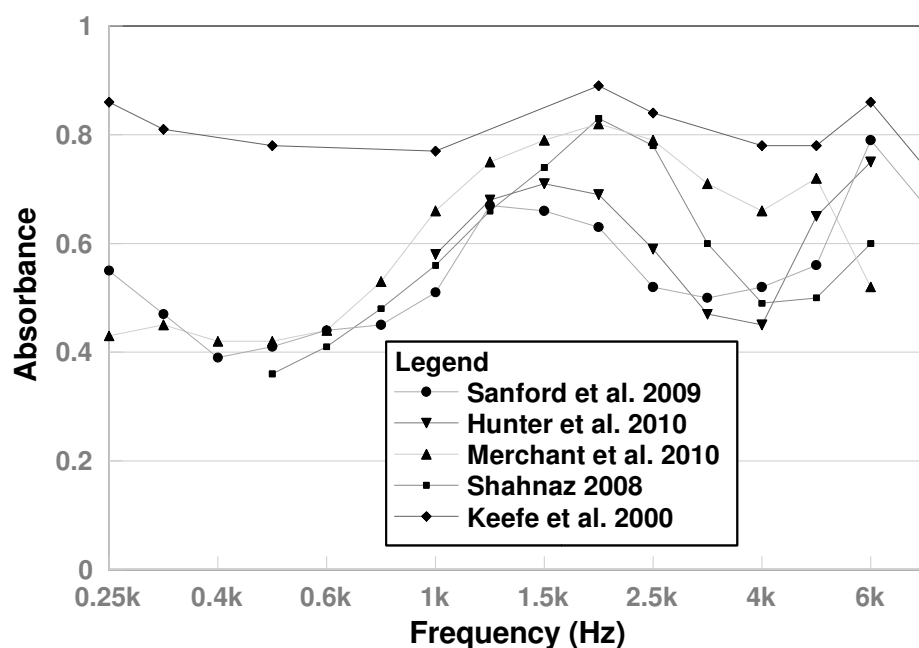


Figure 1.2: Comparison of median wideband absorbance in neonates across five studies

Subsequent studies have reported WBA data from a population of normal hearing healthy full term neonates (Sanford et al., 2009; Hunter et al., 2010; Merchant et al., 2010; Silva, Urosas, Sanches, & Carvallo, 2013) and evaluated its usefulness in relation to NHS programs. Sanford et al. (2009) were the first to report WBA in

relation to NHS and 1000 Hz tympanometry outcomes from a large number of neonates in a well baby nursery. They reported WBA data on 375 ears of healthy, full term neonates who passed DPOAE screening and 80 ears of neonates who failed DPOAE hearing screening during the first two days of life. Median absorbance in ears that passed DPOAE on day 1 varied between 0.39 and 0.67 while that in ears that failed DPOAE varied between 0.20 and 0.40 with the best separation between the two groups observed at 1400 to 2500 Hz (Figure 1.3). Ears that passed DPOAE screening had higher absorbance compared with those that referred indicating that neonates who passed the DPOAE UNHS had a more acoustically efficient conductive pathway.

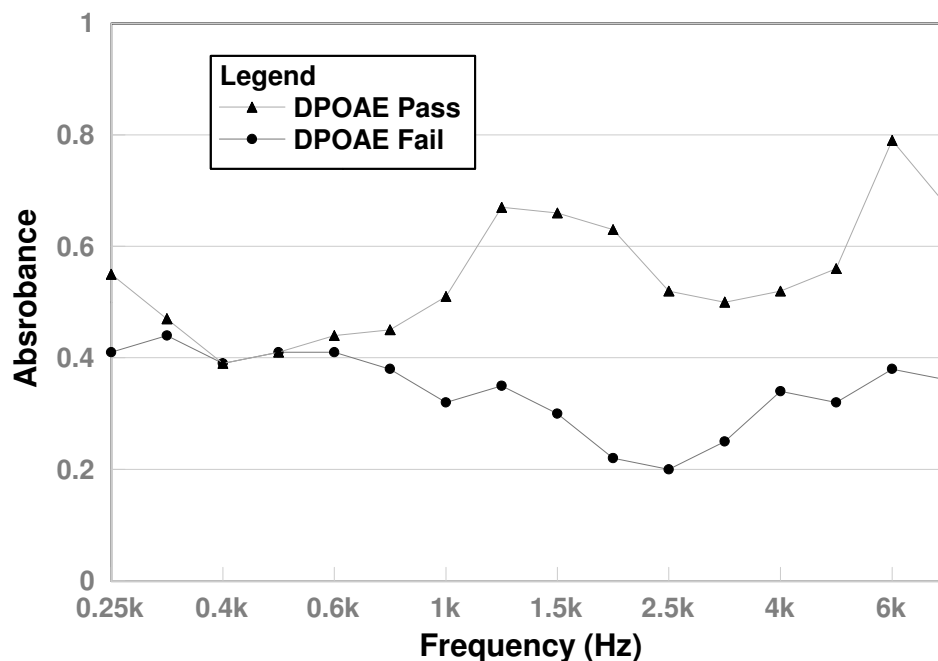


Figure 1.3: Median absorbance in neonates who passed (n = 375) or failed (n = 80) DPOAE screening test in Sanford et al. (2009) study

Similarly, Hunter et al. (2010) investigated WBA in relation to NHS and 1000 Hz tympanometry outcomes from a large number of healthy neonates. The investigators developed normative data for WBA between 1000 and 6000 Hz in 352 neonates who passed DPOAE screening and compared it with 141 neonates who failed the DPOAE screen. Hunter et al. defined the WBA normative region to be between 0th percentile of the DPOAE pass group and 10th percentile of the DPOAE refer group. They also developed area indices wherein the absorbance values were

integrated or averaged over a specified frequency range. Test performance analyses demonstrated that the regions involving 2000 Hz (1000 to 2000 Hz, 1000 to 4000 Hz and 2000 Hz alone) provided the greatest discrimination between DPOAE pass and refer groups. There were no significant ear or gender effects.

Table 1.1: Summary of studies that have investigated WBA under ambient pressure conditions in neonates

Study	Method	Subjects	Summary of findings
Keefe et al. (2000)	WBA measured in well babies without risk indicators, with at least one risk indicator and NICU graduates	2081 neonates divided into 3 groups	Median absorbance of 0.8 across all frequencies. Significant ear and gender effects present Variation of WBA with age present in the first few days of life.
Keefe et al. (2003b)	Retrospective analysis of WBA in relation to TEOAE, DPOAE and AABR	1405 neonate ears	High frequency absorbance was the most important factor in classifying OAE results that classified OAEs with ROC of 0.79 and ABR of 0.64.
Keefe et al. (2003a)	Developed model for middle ear dysfunction	2638 ears used to construct the model	High frequency absorbance was the best predictor (AROC = 0.87).
	Model applied to a different neonate group	1027 normal hearing ears.	Inclusion of this model decreased false positive from 5% to 1.1%.
Vander Werff et al. (2007)	Infants tested during screening and diagnostic testing.	127 infants aged 2 weeks to 24 months screening group – n = 61	Smaller test-retest differences for the diagnostic group Test-retest differences largest for frequencies below 500 Hz and smallest in the mid-frequency range.

Study	Method	Subjects	Summary of findings
		Diagnostic group- n = 66	No difference in test-retest performance between infants who passed or failed OAE screening.
		Control group – 10 normal	Low WBA from 630 to 2000 Hz in infants who failed OAE screening
Hunter et al. (2008a)	Ears classified as normal or poor ear status using otoscopy, tympanometry and DPOAE	97 (194 ears) infants and children aged 3 days to 47 months 3 days - 2 months n = 18 3 - 5 months n = 15 6 - 11 months n = 25 12 - 23 months n = 20 24 - 47 months n = 19	Low absorbance in ears with poor ear status. WBA significantly different between 1000 and 3000 Hz for normal ears
Shahnaz (2008)	Inclusion criteria- NICU babies with pass in both TEOAE and AABR	54 ears (49 pass, 5 fail) from 31 NICU babies 56 adults (age 18 – 32 years) with normal hearing and pass in TEOAE	Clear separation of absorbance between NICU babies and adults below 727 Hz. Absorbance high in NICU babies
Sanford et al. (2009)	Test performance of WBA and 1000 Hz tympanometry used to predict DPOAE outcomes	455 ears (375 pass and 80 fail DPOAE)	Maximum absorption from 1200 to 2700 Hz in normal NICU babies and from 2800 to 4800 Hz in adults AROC 0.87 for ambient WBA and 0.75 for 1000 Hz tympanometry High absorbance in ears with DPOAE pass Best separation of WBA between pass and fail groups from 1400 to 2500 Hz
Hunter et	Test	493 ears from	Normative data provided for

Study	Method	Subjects	Summary of findings
al. (2010)	performance of WBA and 1000 Hz tympanometry used to predict DPOAE outcomes	324 neonates – 352 passed and 141 referred with DPOAE screening	1000 to 6000 Hz frequency range and for absorbance area indices. 2000 Hz was best predictor of DPAOE outcome AROC high for WBA (0.90 and 0.82 at 2000 and 1000 Hz respectively) and 0.72 for 1000 Hz tympanometry. Absorbance differed significantly as a function of DPOAE status and frequency.
Merchant et al. (2010)	Only infants that passed DPOAE screening included	12 ears from seven neonates 19 ears from 11 1-month old infants	Absorbance similar in both groups at most frequencies
Pitaro (2013)	Otoscopy and WBA done on healthy neonates Ear canal occlusion given on a scale of 0 to 100%.	156 neonates	Absorbance significantly different between 0 to 70% and 80 to 100% occlusion groups Significant decrease in absorbance with 70 to 80% occlusion of ear canal diameter
Silva et al. (2013)	Neonates with TEOAE and tympanogram included	144 ears from 77 infants	Normative WBA data provided Energy absorbance less from 250 to 750 Hz and high from 1000 to 3000 Hz

Merchant et al. (2010) studied WBA in 12 ears from seven neonates who passed DPOAE and found that the power absorbance was minimum (near 0.4) at 500 Hz and increased with frequency until about 2000 Hz where it reached a maximum of 0.82. Above 2000 Hz, power absorbance decreased with frequency. There was no difference in absorbance between males and females. There were small, albeit significant, differences between mean absorbance measures of right and left ears.

In a recent study, Silva et al. (2013) studied 77 neonates who passed TEOAE. At low frequencies (250 to 750 Hz) less energy absorbance was observed while at mid frequencies (1000 to 3000 Hz) greater energy absorption was observed. There was no significant difference between ears or between genders.

In addition, several studies have measured ASR including WBA and admittance (Feeney & Keefe, 2001; Feeney & Sanford, 2005). Keefe, Fitzpatrick, Liu, Sanford and Gorga (2010) studied wideband ASR in 455 ears of 230 infants aged one to two days passed or referred a DPOAE based NHS test. They found that an optimal combination of WBA and ASR tests performed better than either test alone in predicting NHS outcomes, and wideband tests performed better than 1000 Hz tympanometry.

Overall, the above studies have provided evidence that WBA could be used to detect middle ear dysfunction and interpret screening results in neonates. However, a limitation of these studies is lack of an easily accessible tool to confirm presence of conductive hearing loss during the neonatal screening period (Hunter et al., 2013). Most studies circumvent this issue by using DPOAE as a gold standard measure to determine normal middle ear status in healthy neonates since it is already used in screening programs (Sangster, 2011). Nonetheless, presence of OAE alone does not rule out effusion or abnormal pressure in the middle ear (Driscoll et al., 2000; Kemp, 2002). Despite using DPOAE as a gold standard in their studies, Sanford et al. (2009) and Hunter et al. (2010) conceded that the presence of DPOAE alone could not be considered as a gold standard measure for middle ear function because DPOAEs may be present despite minor middle ear dysfunction. Use of OAE as a gold standard represents a significant shortcoming and limits the clinical applicability of WBA in neonates (Sangster, 2011). Therefore, further WBA research needs to consider more

robust gold standards such as composite of tests to assess the clinical effectiveness of WBA.

1.9.1.6 Developmental trends in WBA.

Studies that have investigated developmental changes in the first few months of life have found higher absorbance at low and mid frequencies compared to adults but similar absorbance at high frequencies (Feeney & Sanford 2005; Keefe et al., 1993; Werner et al., 2010). Keefe et al. (1993) was the first to investigate the developmental effects of middle ear maturation on WBA measures. They studied energy absorbance in 10 adults and 78 healthy infants aged 1 to 24 months across five age groups (1, 3, 6, 12 and 24 months) and found systematic changes in absorbance with increasing age. Keefe et al. found that the middle ear compliance was lower and middle ear resistance higher in infants than in adults. The researchers also found a significant effect of age below 1000 Hz with a decrease in absorbance from birth to about six months of age where WBA decreased by 30% with a smaller difference in the mid frequency range. They attributed the age effect to power loss from flaccid canal wall motion apparent in newborns. Werner et al. (2010) studied WBA in 174 two to three month olds and 205 five to nine month olds. They found age related changes during infancy similar to that of Keefe et al. Werner et al. found that the absorbance was slightly higher in the left ear than the right ear. However, they did not find any gender differences.

Sanford and Feeney (2008) reported power absorbance from 60 infants with 20 each at 4, 12 and 27 weeks of age and found the results at ambient pressure condition to be consistent with Keefe et al. (1993). Sanford and Feeney (2008) too found a 30% change in the mean energy absorbance for frequencies from 250 to 750 Hz. The frequency region from 750 to 2000 Hz was reported to be a developmentally stable frequency range with few age related changes. Figure 1.4 compares WBA in infants at three age intervals across studies by Sanford and Feeney (2008) and Keefe et al (1993).

In their study with five infants aged six weeks and three adults, Feeney and Sanford (2005) reported that significantly higher absorbance was observed at all frequencies for the infants compared to adults, and the difference was greatest in the low frequency region. Contrary to the above findings, Hunter et al. (2008b) found no

significant age effect with respect to absorbance in their study population. The investigators studied 159 ears from 81 children aged 3 days to 47 months and found no significant age effect across age groups from 250 to 8000 Hz except at 6000 Hz. Although some variability occurred across age groups, there was no apparent systematic effect and the 95 percent confidence intervals overlapped from birth to four years of age. Merchant et al. (2010) studied 12 ears of seven healthy neonates and 19 ears of 11 one month old infants. Except for a slight difference at 2000 Hz between neonates and one month olds, there was no difference in power absorbance between the two age groups and between males and females across all other frequencies. There were small differences in some frequency bands between right and left ears. Hunter et al. and Merchant et al. suggested that the reason they did not find developmental changes compared to other studies could be partly attributed to methodological differences including the age of subjects and differences in the equipment and probe tips.

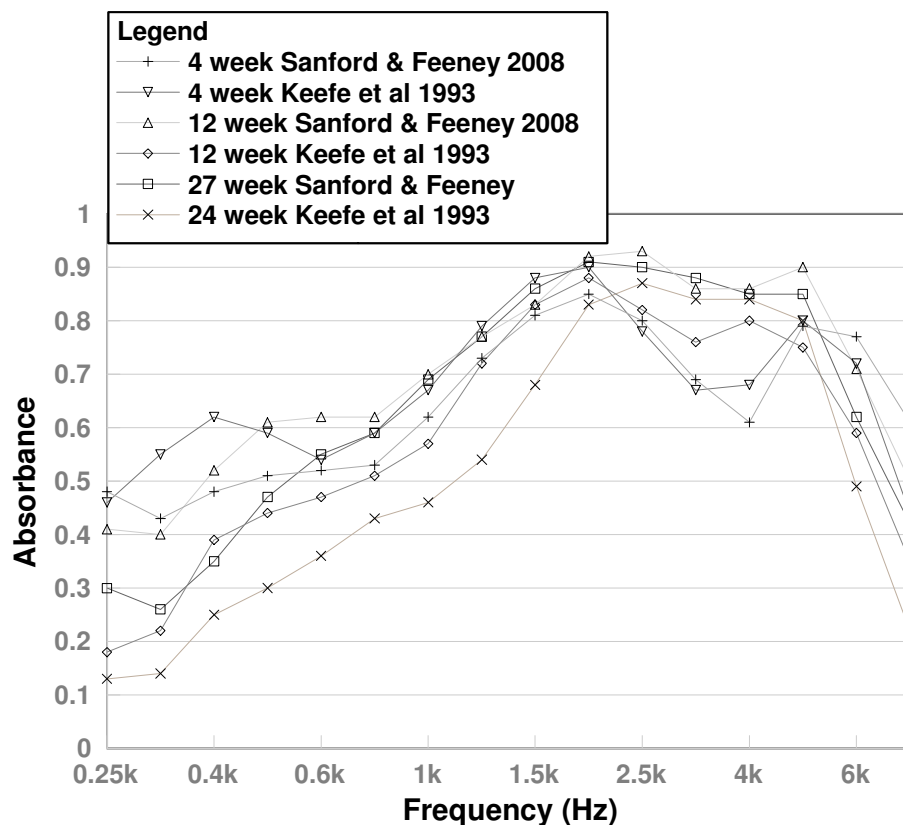


Figure 1.4: Wideband absorbance of infants aged 1 to 6 months in studies by Sanford & Feeney (2008) and Keefe et al. (1993)

In a recent study, Shahnaz, Cai and Qi (2014) established normative WBA data in infants from birth to six months of age (Figure 1.5). Using a longitudinal paradigm, they also studied the time course and rate at which functional maturation of the middle ear occurs in 18 infants from birth through to six months of age. They found that WBA decreased (closer to 0 at low frequencies (<400 Hz) and increased (closer to 1) at high frequencies (>2000 Hz) as a function of age. There was very little change in power absorbance from 600 to 1600 Hz across the first six months of life.

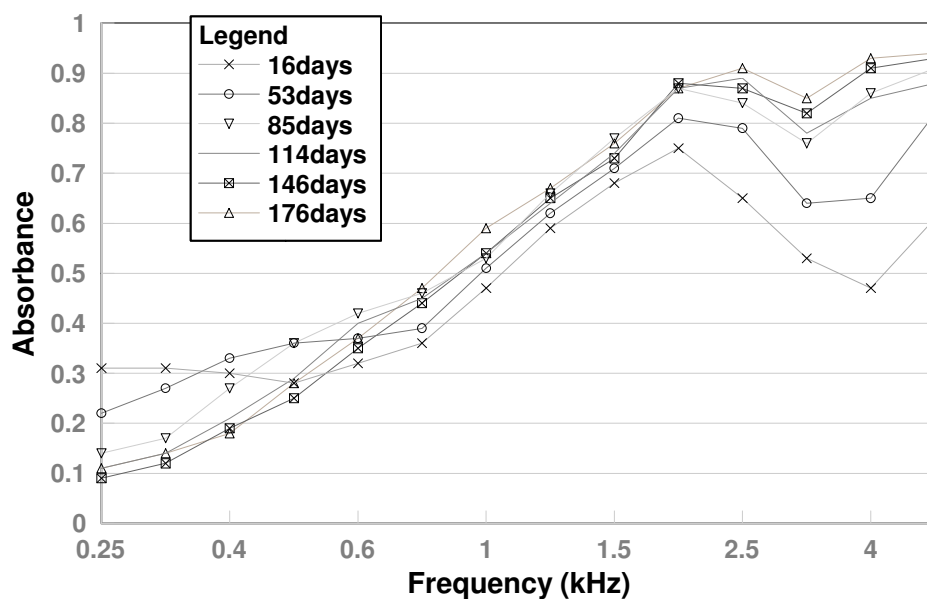


Figure 1.5 Mean absorbance as a function of frequency during six evaluations from birth to six months of age in a longitudinal study by Shahnaz et al. (2014)

To sum up, investigators who have found developmental changes to WBA recommend the range of frequencies up to 2000 Hz as the frequency region of interest since infants have shown a significant difference in this frequency range (Hunter et al., 2010; Keefe et al., 1993; Sanford & Feeney, 2008). Currently, normative data, especially for the first six months of life, has been limited, as the age range covered by several studies beyond the newborn period is restricted. In addition, there has been no agreement regarding the developmental changes in WBA. This lack of agreement can partly be attributed to the methodological differences including the different age groups and equipment across the studies. Due to significant developmental changes during early infancy, there is a need to include infants at various age intervals in order to more closely describe developmental effects on the outer and middle ear (Sanford

& Feeney, 2008). Age-graded norms are essential to the successful clinical application of WBA measures, especially in the period from newborn to one year of age (Hunter et al., 2013).

1.9.2 Measuring WBA under tympanometric pressure conditions

A more complicated procedure, known as wideband tympanometry (WBT), measures WBA as the pressure in the ear canal is varied. In this procedure, clicks are presented to the ear canal and WBA is measured across the frequency range from 250 to 8000 Hz when the ear canal pressure is varied from +200 to -300 daPa. Figure 1.6 shows the typical wideband tympanometric pattern with a three-dimensional representation of absorbance plotted as a function of pressure and frequency.

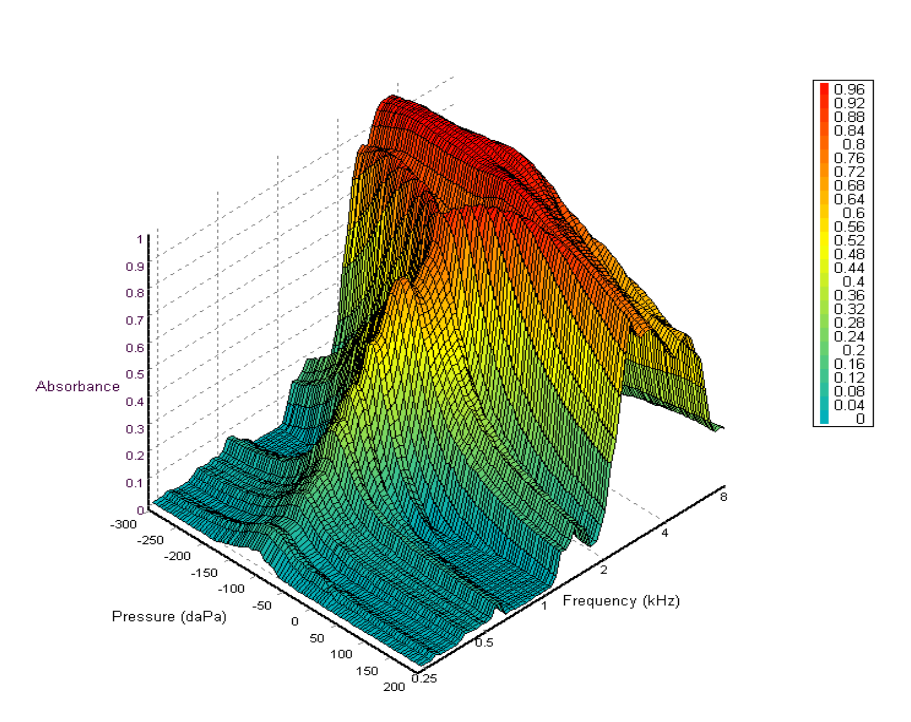


Figure 1.6: Wideband tympanometric plot from left ear of a 7-year-old child

Research has shown WBT to be equally or more sensitive to middle ear dysfunction compared to WBA measured under ambient pressure conditions (Beers et

al., 2010; Ellison et al., 2012; Keefe et al., 2012; Keefe & Simmons, 2003; Margolis et al, 1999; Sanford et al., 2009). Margolis et al (1999) obtained WBT results from 20 adults and demonstrated that WBT pattern progressed in an orderly fashion as frequency increased from 2000 to 11000 Hz. They suggested that WBT can be a useful clinical measure since it is sensitive to middle ear dysfunction and demonstrates systematic variation across a wide frequency range.

The test performance of WBT during assessment of middle ear status in children and adults is reported to be either similar or higher than WBA at ambient pressure (Margolis et al., 1999; Keefe & Simmons, 2003; Keefe et al., 2012; Sanford, Hunter, Feeney & Nakajima, 2013). For instance, Margolis et al (1999) compared absorbance results obtained from a 10-year-old boy with recurrent OM under ambient pressure and pressurised conditions. Surprisingly, they found normal results obtained under ambient pressure, but abnormal WBT results. Keefe and Simmons (2003) evaluated the test performance of WAI under ambient and pressurised conditions and 226 Hz tympanometry in predicting the presence of conductive hearing loss, based on an air-bone gap of ≥ 20 dB in 42 normal ears and 18 ears with conductive or mixed hearing loss. Results showed an AROC of 0.28 for 226 Hz tympanometry, 0.72 for ambient wideband absorbance and 0.94 for WBT.

Keefe et al. (2012) evaluated the test performance of absorbance under ambient and pressurised conditions and 226 Hz tympanometry in terms of their ability to predict conductive hearing loss in 25 children (36 ears) aged 3.5 to 8.2 years with 23 children (44 ears) aged 2.6 to 8.2 years serving as normal controls. They found that both absorbance tests accurately predicted the presence of conductive hearing loss in children, and each was a better predictor of conductive hearing loss than conventional 226 Hz tympanometry. There was no significant difference between the accuracy of ambient and pressurised absorbance measures in detecting conductive hearing loss. Ears with conductive hearing loss had reduced absorbance at frequencies between 700 and 8000 Hz.

Although the effects of ear canal pressure on absorbance in adults (Margolis et al., 1999) and infants (Sanford & Feeney, 2008) are well understood, there is less understanding regarding the effects of introducing ear canal pressure in neonate and young infants (Feeney & Sanford, 2012). Neonatal studies have often measured WAI

under ambient pressure conditions (Keefe et al, 2000; Shahnaz, 2008; Sanford et al, 2009; Hunter et al, 2010; Merchant et al, 2010; Prieve et al, 2013). To date, only one study by Sanford et al. (2009) has investigated WBT in neonates. Sanford et al. (2009) evaluated test performance of ambient and tympanometric measures and 1000 Hz tympanometry in relation to outcomes of distortion product otoacoustic emission (DPOAE) in 455 neonate ears (375 passed, 80 referred). Sanford et al. measured log likelihood ratios of the WAI measures to indicate whether a response from an individual ear was from either the pass or refer group. The highest areas under the curves were 0.87 for ambient WAI test, and 0.84 for WBT compared with 0.75 for 1000 Hz tympanometry

Sanford and Feeney (2008) investigated WBT measurements at varying static ear canal pressures in 4-, 12- and 24- week-old infants and young adults and found developmental changes in WBT measures that varied as a function of frequency. There was as much as a 30% change in mean absorbance at frequencies from 250 to 750 Hz with changes in static ear canal pressure from +200 to -200 daPa. Minimal developmental differences in absorbance were observed at frequencies from 750 to 2000 Hz with changes in ear canal pressure suggesting a developmentally stable frequency range. A high frequency effect between 2000 and 6000 Hz was observed only in 4-week old infants wherein negative pressures caused decreased absorbance and positive pressures caused increased absorbance. Sanford and Feeney suggested that some of the age related effects of pressure on absorbance may be the result of more compliant ear canal walls or less rigid coupling of the ossicles which became more resistant to changes in pressure with age.

In view of the potentially useful diagnostic information provided by WBT in the evaluation of middle ear status in children and adults, WBT could be explored as a technique specifically in neonates and young infants where there is a need for improved assessment techniques of middle ear function (Sanford et al., 2013).

1.9.3 WBA findings in ears with conductive hearing loss and middle ear pathology.

In the last decade, WBA has been used to assess middle ear pathology in children and adults. WBA measurements have been reported in infants and children

with OME (Beers et al., 2010; Ellison et al., 2012; Hunter et al., 2008a; Jeng et al., 1999; Margolis et al., 2000), as well as in adults with otosclerosis, ossicular discontinuity and perforation of the tympanic membrane (Allen et al., 2005; Feeney et al., 2003; Shahnaz et al., 2009), and in older children and adults with conductive hearing loss (Keefe et al., 2012; Keefe & Simmons, 2003; Piskorski et al., 1999).

Keefe and Simmons (2003) studied the test performance of WBA and 226 Hz tympanometry in predicting the presence of conductive hearing loss based on an ABG of 20 dB or more. Subjects included adults and children of age 10 years and older with 42 normal functioning ears and 18 ears with a conductive hearing loss. They found that the absorbance from a conductive impaired ear differed markedly from that of a normal ear. The absorbance of a conductive impaired ear had lower values with increasing frequency compared to a normal ear. The absorbance value in a conductive loss ear rarely exceeded 0.4, while in a normal ear, the absorbance value was approximately 0.9 at the 3000 Hz region. Low absorbance was associated with low transfer of energy into the middle ear and, thus, was consistent with a conductive hearing loss. Comparing tests at a fixed specificity of 0.90, the sensitivity was 0.28 for the static admittance at 226 Hz and 0.72 for the ambient pressure WBA measure. Keefe and Simmons concluded that the ambient pressure WBA measure had sufficient accuracy to be utilised in hearing screening applications. This is in agreement with the findings of Piskorski et al. (1999) who found that a multivariate analysis of admittance-absorbance responses yielded an output that successfully predicted the presence of conductive hearing losses with AROC values as large as 0.97. When the multivariate test performance was assessed at a fixed sensitivity of 0.9, the specificity was as high as 0.94.

In a sample of 97 children ranging from 3 days to 47 months, Hunter et al. (2008b) demonstrated a significant difference in WBA measures in ears clinically defined as having poor ear status (i.e., OME) that was determined by the combined test algorithm of otoscopy, tympanometry and DPOAE. They found that the poor ears had decreased absorbance in the frequency range of 1000 to 4000 Hz compared to normal ears. Jeng et al. (1999) studied three children with OME and reported that ears with OME showed dramatically less power absorption at all frequencies and close to zero absorption below 1000 Hz. Although, there was a substantial increase

in the power absorption above 1000 Hz, the peak absorption was still less than that of normal ears. The largest difference between the normal ears and ears with OME occurred in the region between 1000 to 2500 Hz.

Hunter et al. (2008a) found that the average WBA in infants and children with cleft lip and palate was substantially lower than the normative sample between 1000 and 4000 Hz. The largest difference occurred at about 2000 Hz where the absorbance averaged 77% in healthy children, compared to 0% in children with cleft palate. Pneumatic otoscopy, conventional low frequency tympanometry and 1000 Hz tympanometry findings were abnormal, respectively in 58%, 67% and 73% of 34 ears from 17 children with cleft palate. In contrast, OAE and WBA findings were abnormal in 88 and 82% of the ears, respectively. The overall agreement between the OAE and 1000 Hz tympanometry results was 80%, while the overall agreement between the OAE and WBA results was 88% (Hunter et al., 2008a). WBA, thus, holds promise as an effective tool in detecting middle ear problems in this population.

Ellison et al. (2012) compared the accuracy of WBA to the current clinical guidelines of using pneumatic otoscopy to diagnose MEE. WBA measures were obtained in 53 ears of 44 children prior to confirmation of MEE via myringotomy as the gold standard. These results were compared to an age matched normative group of 59 ears of 44 healthy children serving as controls. The results showed that absorbance was reduced in ears with MEE compared to the control group especially between 800 and 2000 Hz. Absorbance varied systematically with TM mobility based on data from pneumatic otoscopy. The authors concluded that absorbance was sensitive to middle ear stiffness and MEE and that WBA predictions of MEE are as accurate as those reported for pneumatic otoscopy. In comparison, Prieve et al. (2013) studied WBA, high frequency tympanometry using 678 and 1000 Hz tones, and air and bone conduction ABR in 84 ears from 70 infants with a median age of 10 weeks. They found that conductive hearing loss could be accurately detected using WBA, 678 Hz and 1000 Hz tympanometry. Keefe et al. (2013) studied WBA under ambient and pressurized conditions in 26 children without conductive hearing loss (control group) and 24 children with conductive hearing loss (experimental group). They found that absorbance was lower at frequencies above 700 Hz in the conductive hearing loss

group than the control group. WBA in both ambient and tympanometric tests were significantly better predictors of conductive hearing loss than 226 Hz tympanometry.

In summary, the above studies have shown that WBA is well suited to assess conductive conditions in infants and children. Since the WBA is able to assess the conductive pathway (outer and middle ear) independent of the inner ear status, it could be utilised along with hearing screening tools (e.g., AABR) to differentiate conductive from sensorineural hearing loss in young infants.

1.9.4 WBA in UNHS programs.

As the WBA provides reliable information on middle ear function in young infants, it could be a useful tool in UNHS programs. Keefe et al. (2003b) performed a retrospective analysis of DPOAE, TEOAE, ABR and WBA in 2766 ears. Analysis of results revealed that the high frequency absorbance was the most important factor in classifying DPOAE. The odds ratio for high frequency absorbance was 2.44 (95% CI 2.09 – 2.86), suggesting that ears with reduced absorbance in the high frequencies had a higher likelihood for middle ear dysfunction. Keefe, Gorga, Neeley, Zhao and Vohr (2003a) further examined if WBA could be added to a screening battery to improve the prediction of sensorineural hearing loss as later assessed by behavioural audiometry at eight to 12 months of age. They found that WBA and OAE variables combined were better at predicting outcomes than OAE alone. This suggested that inclusion of WBA measures improved prediction of sensorineural hearing loss with DPOAE measurements. High frequency absorbance was the best predictor of middle ear function with an AROC of 0.86 in classifying normally hearing ears as having normal middle ear function and those that fail both tests as having middle ear dysfunction. Inclusion of WBA in to the model, revealed that of the 51 of 1027 ears that failed two stage DPOAE and ABR screening, 40 ears had middle ear dysfunction, thereby reducing false positives from 5% to 1.1%. Therefore, the researchers concluded that inclusion of WBA in addition to OAE could enhance the ability to predict hearing status and improve the accuracy of newborn hearing screening programs.

Sanford et al. (2009) investigated WBA in 455 neonate ears in a UNHS program using DPOAE as a screening tool. They found that the 375 ears that passed

the DPOAE test had higher absorbance than the 80 ears that were referred, indicating that neonates who passed DPOAE had a more acoustically efficient conductive pathway. Sanford et al. also found that WBA had better performance in classifying DPOAE outcomes than 1000 Hz tympanometry. Using DPOAE as a reference standard, Sanford and colleagues found that the greatest AROC value was 0.87 for WBA and 0.75 for 1000 Hz tympanometry. They concluded that WBA results in ears that were referred from UNHS were related to transient conditions affecting the sound conduction pathway and that WBA is sufficiently objective, quick, and feasible to consider implementing in conjunction with the UNHS program.

Hunter et al. (2010) reported WBA data in a newborn hearing screening population and compared WBA test performance with 1000 Hz tympanometry for predicting DPOAE screening outcome in 324 infants. Hunter et al. showed that WBA measured in a frequency region around 2000 Hz provided the best prediction of DPOAE outcomes and that WBA produced better prediction of DPOAE status than 1000 Hz tympanometry. The authors also observed that WBA improved significantly during the first four days after birth with normalization of middle ear function. They recommended that neonates with low absorbance scores during the first screen should be rescreened within a few hours to a few days, because most transient conductive conditions may resolve spontaneously. If absorbance and OAE are not passed upon second screening, they suggested referral to an otologist for ear examination along with diagnostic testing. They further suggested that neonates who failed in both WBA and OAE should be referred to an otologist for ear examination along with diagnostic testing, while neonates who passed WBA but failed OAE should be referred immediately to an audiologist for diagnostic testing with threshold auditory brainstem response because of higher risk of permanent hearing loss.

Although the above mentioned studies compared WBA with OAE results, OAE may not be an ideal reference standard because OAEs may be present despite minor middle ear dysfunction (Hunter et al., 2010; Kemp, 2002; Sanford et al., 2009). This may call for further WBA research using a stronger reference standard, such as a combination of diagnostic measures to separate healthy ears from ears with a conductive condition.

1.9.5 Reliability of WBA.

As WBA is an emerging technology, its reliability needs to be ascertained before it can be used as a clinical tool with infants. Studies evaluating test-retest reliability of WBA in infants have reported good reliability (Hunter et al., 2008a; Shahnaz et al., 2014; Vander Werff et al., 2007). In the study by Vander Werff et al. (2007), test-retest reliability of the WBA measure obtained from an infant group (N = 61) receiving outpatient hearing screenings (mean age = 7.6 weeks, SD=5.3) was compared to that obtained from another infant group (N = 66) receiving outpatient diagnostic assessments (mean age = 12.4 weeks, SD= 8.5 weeks). Better test-retest reliability was reported for the diagnostic than for the screening group. The difference in reliability was attributed to decreased noise in the testing environment for the diagnostic group. Hunter et al. (2008a), in their study of infants with unrepaired cleft lip and palate, found substantial reliability of repeat measures of WBA within the same test session even after removal and reinsertion of the probe tip. They also found that the reliability results were equivalent irrespective of the type of stimuli (broadband chirps and specific sine waves) used for the WBA measure.

Despite its high test-retest reliability, a potential limitation of WBA appears to be the variability of WBA results. Hunter et al. (2008b) reported a high degree of variability in WBA in healthy ears and ears with OME. They attributed the sources of variance to the internal noise in the ear canal, ambient noise in the test room, depth of insertion of the probe tip, probe seal and calibration of the probe. In fact, several studies have highlighted the importance of a leak-proof seal in order to obtain accurate measurements and avoid variability in the results (Hunter et al., 2008a; Keefe et al., 2000; Vander Werff et al., 2007).

1.10 Rationale for the Study

1.10.1 Synopsis.

False positive referrals due to outer and middle ear dysfunction continue to be an issue with the newborn hearing screening programs. In order to reduce the false positive referrals, prioritise infants for testing and alleviate parental anxiety, a reliable and objective measure that assesses the outer and middle ear system of infants at the time of screening is warranted. WBA holds promise as an objective tool for the assessment of conductive conditions in neonates and young infants. It has been

recommended for use along with screening tools in UNHS programs. Before WBA can be used as a clinical tool with screening or diagnostic evaluation, normative data need to be developed and the test performance must be evaluated against stringent reference standards. Currently, there is limited research in the application of WBA to infants in Australia. Further Australian studies using WBA in infants are required in view of the high prevalence of OM in Australian Aboriginal infants.

1.10.2 Justification for conducting the present study.

A review of the literature has identified some issues regarding the use of WBA with neonates and young infants. The present study seeks to address five of these issues. First, although newborn hearing screening programs have been introduced in Australia for more than a decade, there are no reports on the false positive rates as well as prevalence of conductive hearing loss in the young infant population. It is important to determine the prevalence of conductive hearing loss in both Australian Aboriginal and non-Aboriginal infants referred from screening programs to determine if there is a need for an adjunct tool for middle ear assessment in this population.

Second, the WBA is reported to have advantage over other measures of middle ear assessment in infants (Keefe et al., 2000). Recent studies have shown that WBA measures perform better in classifying the DPOAE outcomes at birth than 1000 Hz tympanometry (Hunter et al., 2010; Sanford et al., 2009). Due to its ability to assess the outer and middle ear independent of the inner ear, it is also suggested as a feasible tool to be used in conjunction with UNHS programs (Keefe et al., 2003a; Sanford et al., 2009). To date, WBA has been described in infants who were assessed by DPOAEs only (Hunter et al., 2010; Merchant et al., 2010; Sanford et al., 2009). However, DPOAE may not identify minor or sub-clinical middle ear pathologies (Kemp, 2002) and hence may not be an ideal gold standard (Hunter et al., 2010; Sanford et al., 2009). While a single measure such as DPOAE or 1000 Hz tympanometry may not be sensitive to subtle middle ear dysfunction, a test battery may provide a robust measure of middle ear function to evaluate the WBA. Nevertheless, normative WBA data based on a set of clinical tests including TEOAEs and 1000 Hz tympanometry have not been investigated. The present study will develop normative WBA measures in healthy neonates that pass a battery of tests that includes AABR, 1000 Hz tympanometry, acoustic stapedial reflex, TEOAE and

DPOAE. A strict gold standard would reduce the possibility of mild or sub-clinical middle ear conditions being included in the normative population.

Third, the test performance of WBA in assessing the conductive pathway in neonates and young infants was found to be higher than that of 1000 Hz tympanometry (Hunter et al., 2010; Merchant et al., 2010; Sanford et al., 2009). However, the use of DPOAE as a reference standard did not yield optimal results. This represents a significant shortcoming in the evaluation of WBA, and limits its clinical applicability (Sangster, 2011). Since there is no easily accessible and accurate tool to detect conductive hearing loss during the neonatal screening period, an alternate, albeit less strict, reference standard would be a composite reference standard involving a battery of tests (Mazlan & Kei, 2012). Nevertheless, an important research question is to determine if the test performance of WBA varies with the choice of a single test (such as DPOAE or HFT) or composite test battery reference standard. The present study seeks to evaluate the test performance of WBA against seven reference standards consisting of single tests and composite test batteries including AABR, HFT, TEOAE and DPOAE in an attempt to determine whether WBA can provide a more effective alternate to either single tests or a combination of tests for determining the outer and middle ear status in neonates.

Fourth, despite the high prevalence of OM in Aboriginal children, there is a lack of studies on assessing the function of their outer and middle ears at birth. Early diagnosis and early intervention of middle ear disease are imperative. Surprisingly, there have been only three studies by Boswell and Nienhuys (1995, 1996) and Lehmann et al. (2008) that have reported otoscopy, 220 Hz tympanometry and TEOAE findings in Aboriginal and Caucasian neonates from birth to two years of age. Nevertheless, these studies have not studied neonates in their first few days of life. Additionally, otoscopy, 220 Hz tympanometry and TEOAE are not sensitive to detecting conductive conditions in neonates and young infants. To date, there have been no studies using 1000 Hz tympanometry or WBA with this young population. The present study is designed to study middle ear function using WBA along with other measures, such as OAE and 1000 Hz tympanometry, in both Aboriginal and Caucasian neonates.

Fifth, if WBA is to be used as a valid clinical tool of outer and middle ear assessment in young infants, developmental variations of WBA need to be described. To date, there has been only one longitudinal study by Shahnaz et al. (2014) that has tracked the developmental WBA pattern during early infancy. However, earlier studies have assessed infants across a wide age range. Hence, age-specific norms are not readily available. In addition, the results of studies on the effect of age on WBA in the first few months of life have been equivocal (Hunter et al., 2008a; Keefe et al., 1993; Merchant et al., 2010). Thus, more research is needed to obtain age-dependent norms and determine the developmental trends of WBA at regular intervals during the first six months of life. The present study will measure WBA in infants at birth and at 1, 2, 4, and 6 months of age to track developmental changes during this fast growing period.

1.11 Aims of the Current Investigation

The current investigation aimed to:

- (1) Evaluate the prevalence of conductive hearing loss and middle ear pathology in infants referred by a NHS program in Australia and compare the prevalence rates of conductive conditions in Aboriginal and non-Aboriginal infants (see chapter 2).
- (2) Obtain normative WBA data in healthy neonates who pass a combination of tests including AABR, HFT, ASR, TEOAE and DPOAE (see chapter 3).
- (3) Compare test performance of WBA using individual measures such as AABR, HFT, TEOAE or DPOAE and a combination of tests (e.g., HFT+DPOAE) to determine whether WBA can provide an effective alternate to either single test or test battery (composite) reference standards for determining the outer and middle ear status in neonates (see chapter 4).
- (4) Compare WBA measures obtained from healthy Aboriginal neonates with that obtained from non-Aboriginal neonates (see chapter 5).
- (5) Conduct a cross-sectional study on a sample of normal infants to determine the developmental trend of WBA results at birth and at 1, 2, 4 and 6 months of age (see chapter 6).

To achieve these aims, the following studies were conducted:

- (1) “Conductive hearing loss and middle ear pathology in young infants referred through newborn universal hearing screening program in Australia” (chapter 2).
- (2) “Normative wideband reflectance measures in healthy neonates” (chapter 3).
- (3) “Wideband absorbance outcomes in neonates: A comparison with high frequency tympanometry, automated auditory brainstem response, transient evoked and distortion product otoacoustic emissions’ (chapter 4).
- (4) “Wideband absorbance in Australian Aboriginal and Caucasian neonates” (chapter 5).
- (5) “Wideband absorbance in young infants (0-6 months): A cross-sectional study” (chapter 6).

1.12 Major Hypotheses of the Present Investigation

This thesis contains four null hypotheses to be tested. They are:

H₀₁: There will be no significant difference in the prevalence of conductive hearing loss between Aboriginal and Caucasian infants who are referred for diagnostic evaluation through a NHS program.

H₀₂: There will be no significant difference in the test performance of WBA between single tests and test battery reference standards.

H₀₃: There will be no significant difference in WBA results between Aboriginal and Caucasian neonates.

H₀₄: There will be no significant age effects on WBA results obtained from infants aged 0- to six months.

Chapter Two: Conductive Hearing Loss And Middle Ear Pathology In Young Infants Referred Through Newborn Universal Hearing Screening Program In Australia

2.1 Background

Although newborn hearing screening programs have been introduced in Australia across all states and territories, there is no data on the prevalence of conductive hearing loss in young infants. Australian Aboriginal children have a high prevalence of conductive hearing loss and middle ear dysfunction that starts from a young age. However, there are no published studies that have documented the prevalence of conductive hearing loss and middle ear dysfunction in Australian and non-Aboriginal infants referred through newborn hearing screening.

Chapter Two provides an account of the conductive disorders in infants referred through a newborn universal hearing screening program in Australia. A retrospective hospital chart review was conducted to analyse the diagnostic test results of infants referred through newborn hearing screening program in north Queensland. In this study, the prevalence of conductive hearing loss in Aboriginal and non-Aboriginal infants are compared.

Chapter Two of this thesis, entitled, “Conductive hearing loss and middle ear pathology in young infants referred through a newborn universal hearing screening program in Australia” has been published in the *Journal of the American Academy of Audiology*. This published article is inserted into this thesis with minor modifications. In particular, the formatting of section sub-headings has been modified from the original publication to match the thesis format.

Aithal, S., Aithal, V., Kei, J., & Driscoll, C. (2012). Conductive hearing loss and middle ear pathology in young infants referred through a newborn universal hearing screening program in Australia. Journal of the American Academy of Audiology 23: 673-685.

2.2 Abstract

Background: Although newborn hearing screening programs have been introduced in most states in Australia, the prevalence of conductive hearing loss and middle ear pathology in the infants referred through these programs is not known.

Purpose: This study was designed to (1) evaluate the prevalence of conductive hearing loss and middle ear pathology in infants referred by a newborn hearing screening program in north Queensland, (2) compare prevalence rates of conductive hearing loss and middle ear pathology in indigenous and nonindigenous infants, and (3) review the outcomes of those infants diagnosed with conductive hearing loss and middle ear pathology.

Research Design: Retrospective chart review of infants referred to the Audiology Department of The Townsville Hospital was conducted.

Study Sample: Chart review of 234 infants referred for one or both ears from a newborn hearing screening program in north Queensland was conducted. A total of 211 infants attended the diagnostic appointment. Review appointments to monitor hearing status were completed for 46 infants with middle ear pathology or conductive hearing loss.

Data Collection and Analysis: Diagnosis of hearing impairment was made using an age-appropriate battery of audiological tests. Results were analysed for both initial and review appointments.

Results: Mean age at initial diagnostic assessment was 47.5 days (SD 31.3). Of the 69 infants with middle ear pathology during initial diagnostic assessment, 18 had middle ear pathology with normal hearing, 47 had conductive hearing loss, and 4 had mixed hearing loss. Prevalence of conductive hearing loss in the newborns was 2.97 per 1,000 while prevalence of middle ear pathology (with or without conductive hearing loss) was 4.36 per 1,000. Indigenous Australians or Aboriginal and Torres Strait Islander (ATSI) infants had a significantly higher prevalence of conductive hearing loss and middle ear pathology than non-ATSI infants (35.19 and 44.45% vs 17.83 and 28.66%, respectively). ATSI infants also showed poor resolution of conductive hearing loss over time with 66.67% of ATSI infants reviewed showing persistent conductive hearing loss compared to 17.86% of non-ATSI infants. Medical management of 17 infants with persistent conductive hearing loss included monitoring, antibiotic treatment, examination under anesthesia, and grommet insertion.

Conclusions: Conductive hearing loss was found to be a common diagnosis among infants referred through screening. ATSI infants had significantly higher rates of middle ear pathology and conductive hearing loss at birth and showed poor resolution of middle ear pathology over time compared to non-ATSI infants. Future research using a direct measure of middle ear function as an adjunct to the automated auditory brainstem response screening tool to distinguish conductive from sensorineural hearing loss may facilitate prioritization of infants for assessment, thus reducing parental anxiety and streamlining the management strategies for the respective types of hearing loss.

Key words: newborn hearing screening, middle ear pathology, conductive hearing loss, neonates, indigenous

2.3 Introduction

Universal newborn hearing screening programs are becoming standard practice in Australia and internationally. Most often and, perhaps, rightly so, high priority is given to early identification and intervention of permanent hearing loss. However, congenital conductive hearing losses (which may be transient or long-standing) are treated as false positive outcomes: a by-product of the screening program.

Conductive hearing loss due to middle ear pathology appears to be common in neonates as studies have reported conductive disorders to be roughly 30 times greater than that of inner ear pathologies in infants (Gorga et al, 2001; Allen et al, 2005). White et al (1993) reported that 17 of 1,000 well infants and 36 of 1,000 infants who had been in the newborn intensive care unit had conductive hearing loss. Boone et al (2005) have pointed out that otitis media with effusion may contribute up to 67% of the false positive newborn hearing screens. Amniotic fluid contents aspirated into the middle ear have been reported to contribute to conductive hearing loss in neonates (deSa, 1977; Eavey, 1993; Northrop et al, 1999).

The only definitive tests for the presence of middle ear effusion (MEE) are myringotomy or imaging studies such as computed tomography; however, neither of these is practical or ethical for the evaluation of infants. Identification of MEE using

otoscopy is not a reliable method in neonates due to difficulties in visualising and interpreting the tympanic membrane (McLennan and Webb, 1957; Zarnoch and Balkany, 1978; Marchant et al, 1984; Eavey, 1993; Doyle et al, 1997; Rhodes et al., 1999). Although otomicroscopy is reported to improve the accuracy of diagnosing otitis media in children (Young et al, 2009; Lee, 2010), it needs to be performed by specialist otolaryngologists and often requires sedation or anaesthetisation of the child.

Apart from AC and BC ABR test, there are no clinically validated tests for diagnosing middle ear dysfunction in infants younger than 6 months (Hunter and Margolis, 1992; Keefe et al, 2003). Conventional 226 Hz tympanometry is not effective in detecting MEE in infants (Paradise et al, 1976; Rhodes et al, 1999). Instead, 1,000 Hz probe-tone tympanometry is recommended with infants under 6 mo of age since this higher frequency appears to be more sensitive than 226 and 600 Hz probe tones for detecting MEE in this age range (Paradise, 1976; Hunter and Margolis, 1992; McKinley et al, 1997; Kei et al, 2003; Margolis et al, 2003). However, a test battery approach is often used to diagnose middle ear dysfunction in infants younger than 6 mo. Studies have suggested the presence of strong transient evoked otoacoustic emissions (TEOAEs) in conjunction with 1,000 Hz tympanometry to be indicative of normal middle ear functioning (Sutton et al, 1996; Kei et al, 2003). Using a combination of otoscopy, acoustic reflex measurements and tympanometry in 68 full term neonates, Roberts et al (1995) reported MEE to be present in all the babies in the first 3 hr of life. By the third day, MEE had resolved in 73% of ears by otoscopy, 88% by acoustic reflex measurements and 92% by tympanometry. Boone et al (2005) used a combination of otoscopy, auditory brainstem response (ABR) and otoacoustic emission (OAE) testing and found that MEE was identified in 64.5% of 76 neonates referred for diagnostic evaluation following referral from newborn hearing screening.

An extraordinary high prevalence of ear disease and hearing impairment among Australian Aboriginal children has been well documented by several cross-sectional studies (Clements, 1968; Dugdale et al, 1978; McCafferty et al, 1985; Foreman, 1987; Kelly and Weeks, 1991; Clarke, 1992; Nienhuys et al, 1994). Several prospective otoscopic and audiological studies of young Aboriginal infants too have

shown a very high prevalence of otitis media ranging from 67 to 95% by 12 mo of age (Douglas and Powers, 1989; Rebgetz et al, 1989; Boswell et al, 1993; Boswell and Nienhuys, 1995). Despite this high prevalence of ear disease, there is only one audiological study (Boswell et al, 1993) and a handful of microbiological studies (Leach et al, 1994; Morris et al, 2009) that document the natural history of MEE and conductive hearing loss from birth in Aboriginal infants.

While statistical data on congenital sensorineural hearing loss in Australian newborns are available (Upfold and Ispey, 1982; Davis et al, 1997; Mehl and Thomson, 1998; Bailey et al, 2002; Australian Hearing, 2005), there are no published studies on the prevalence and natural history of conductive hearing loss in neonates despite the introduction of newborn hearing screening programs across the country. The state of Queensland in Australia offers hearing screening using automated ABR (AABR) for all newborns. This program, called Healthy Hearing, began as a pilot project in three tertiary hospitals in October 2004 and has now become a state-wide program that covers all tertiary and regional hospitals and home births. Despite the successful rollout of the program across the state, there are, as yet, no published data on the prevalence and course of conductive hearing loss and middle ear pathology in infants referred for diagnostic assessment through this program.

The objectives of the present study were to (i) evaluate the prevalence of conductive hearing loss and middle ear pathology in infants referred by a newborn hearing screening program in north Queensland, Australia (ii) compare prevalence rates of conductive hearing loss and middle ear pathology in indigenous and non-indigenous infants and (iii) review the outcomes of those infants diagnosed with conductive hearing loss and middle ear pathology.

2.4 Method

2.4.1 Subjects

Under the Healthy Hearing Program, a total of 15,824 infants were screened during the period from August 2004 to March 2009. Of these infants, 1,836 (11.6%) were indigenous Australians or Aboriginal and Torres Strait Islanders (ATSI), 13,830 (87.40%) were nonindigenous Australians or non-ATSI and 158 (1.0%) were of

unknown ethnic background. The mean age of infants at the time of screening was 23.9 days (SD = 23.64, range = 0.96 days).

The Audiology Department at the Townsville Hospital serves as a tertiary referral centre for the diagnostic assessment of infants referred through newborn hearing screening in north Queensland. A total of 234 infants (63 ATSI and 171 non-ATSI) who did not pass the two tier AABR screening in either one or both ears were referred for diagnostic audiology assessment between August 2004 and March 2009. A retrospective hospital chart review of the medical records was performed by an experienced paediatric audiologist for these 234 infants. This study is a part of a major project on identification of middle ear pathology in infants for which chart review was performed as part of a quality assessment audit of the state-wide hearing screening program. Ethical clearance was obtained from the Townsville Health Service District Institutional Ethics Committee.

2.4.2 Procedure

2.4.2.1 Initial diagnostic assessment.

Figure 2.1 depicts the flow chart for diagnostic and review audiology assessments as per the Healthy Hearing program protocol (Keogh and Beahan, 2009). A battery of audiological tests as per the program protocol was administered by an experienced paediatric audiologist in a quiet non-sound treated room. The minimum test battery included 1,000 Hz probe-tone tympanometry, TEOAEs and click-evoked ABR audiometry. Other audiological tests that were not part of the minimum test battery were not routinely performed but were administered as required to obtain further information on the type and degree of hearing loss. The ambient noise level as measured using a sound level meter (SdB 01) ranged from 33 to 45 dB A.

The infant was considered to have functionally normal hearing with normal middle ear function if results of all the assessments (tympanometry, TEOAEs, and click ABR) were normal in both ears and was subsequently discharged from the program. If an infant was found to have hearing loss or middle ear pathology with normal hearing in one or both ears, another appointment was organised within 7 to 10 days to confirm the findings. In addition to the minimum test battery described above, auditory steady state response (ASSR), 1,000 Hz tone burst ABR and bone

conduction ABR were performed as required. Following the confirmation of hearing loss, infants with sensorineural and mixed hearing loss were referred for amplification, medical investigation and early intervention services.

2.4.2.2 Review Assessment.

Infants diagnosed with middle ear pathology and normal hearing, or with conductive hearing loss or indeterminate hearing loss were referred for review assessment in 6-8 weeks' time. This study did not follow the outcomes of those with indeterminate hearing loss. During this review, each infant underwent a test battery of 1,000 Hz or 226 Hz tympanometry depending on the age, TEOAEs, and click ABR. If hearing loss was found, further assessments using 1,000 Hz tone burst ABR, ASSR, bone conduction ABR and/or visual reinforcement audiometry (VRA) were performed. The same paediatric audiologist carried out both initial and review assessments using the following diagnostic tests.

2.4.3 Diagnostic tests

2.4.3.1. Tympanometry.

Tympanometry was performed using a GSI Tymptstar middle ear analyser. Tympanograms were obtained using a 1,000 Hz probe tone with the pressure varying from 200 daPa to -400 daPa. The pass criteria were repeatable single or notched admittance peaks with compliance ≥ 0.3 mmho and middle ear pressure around 0 daPa (Keogh and Beahan, 2009). Additionally, a 226 Hz probe tone was used when the infants were 6 mo of age or older. The pass criteria were a type A tympanogram as per Jerger's (1970) classification system, with middle ear compliance ≥ 0.3 ml and middle ear pressure between +50 and -150 daPa in both ears (Alaerts et al, 2007).

2.4.3.2 Transient Evoked Otoacoustic Emissions (TEOAEs).

A Biologic Scout (v.3.45) was used to measure TEOAEs. The signal consisted of wide band clicks of 80 μ s duration, at a target amplitude of 80 dB peak Sound Pressure Level (pkSPL). The pass criteria included reproducibility of at least 70%, and a difference between the amplitude of the emission and the associated noise floor of at least 6 dB in at least three out of four frequency bands (1,500, 2,000, 3,000 and 4,000) including 1,500 and 4,000 Hz in both ears (Kemp et al, 1990; Amedee, 1995).

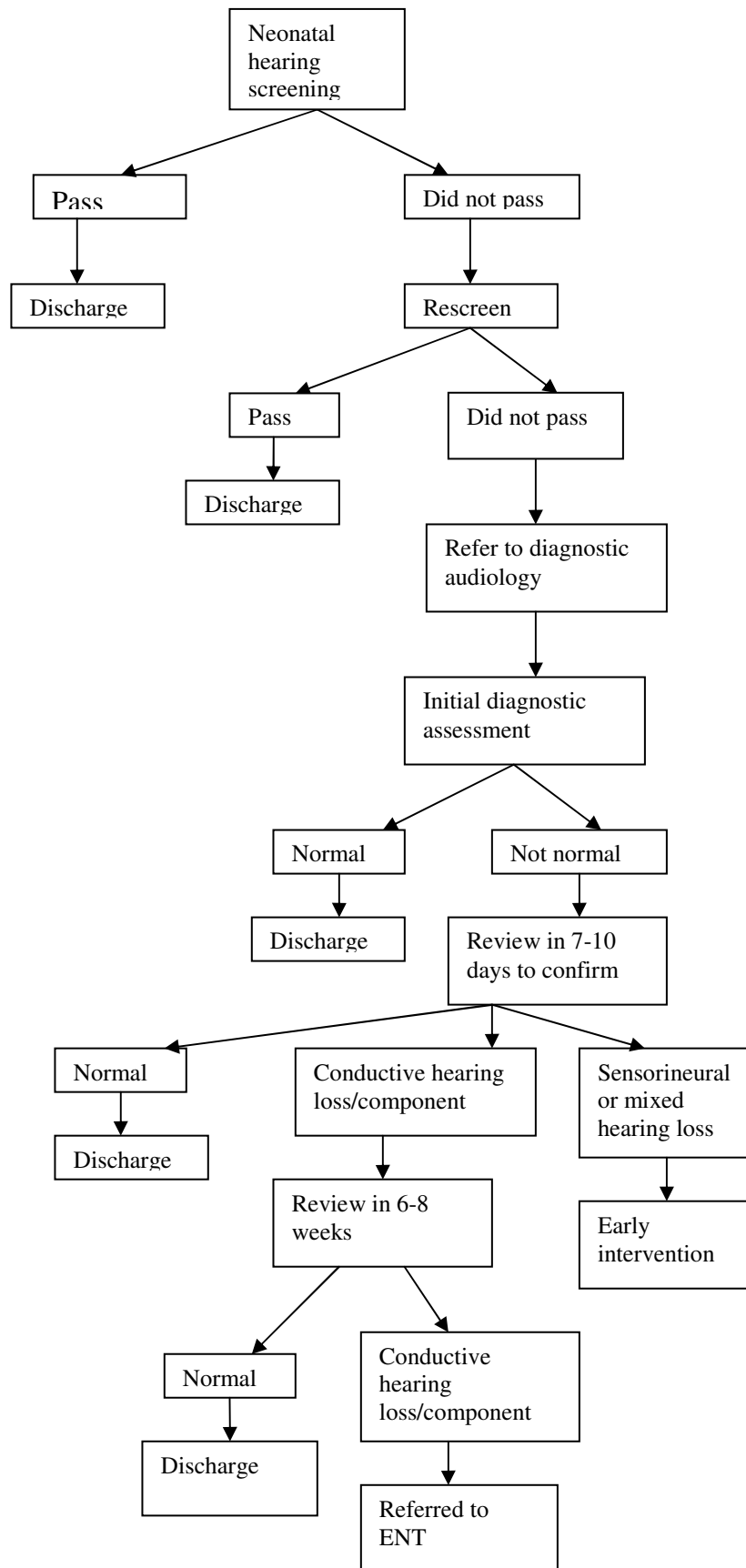


Figure 2.1: Flow chart for diagnostic and review audiology

2.4.3.3 Click-evoked Air conduction ABR

Single channel click ABR recordings were obtained from both ears in sleeping infants using Biologic auditory evoked potentials Navigator Pro System (v.6.2.0). Rarefaction pulses of 100 μ s were delivered at a rate of 27.1/s through insert earphones. Stimuli were presented in descending steps from 80 dB normalised hearing level (nHL) to below response threshold with a maximum of 2,000 stimuli per response. A stimulus level of 70 dB was chosen in some instances when the baby was disturbed by the 80 dB signal level. Thresholds were tracked using latency of wave V in both ears. A minimum of two replications of 2,000 trials each was obtained at 50 dB and below. Additional replications were obtained when necessary to confirm the presence of response. Pass criteria included repeatable wave V at normal latencies at 30 dBnHL, normal intensity-latency function from 80 to 30 dBnHL and normal interpeak latencies at 80 dBnHL (Keogh and Beahan, 2009).

2.4.3.4 1,000 Hz Tone Burst ABR.

Tone burst ABR was performed using the Biologic auditory evoked potentials (v.6.2.0). The response determination criteria were the same as described for click ABR. The 1,000 Hz tone bursts with rarefaction polarity were delivered at a rate of 27.7/s through insert earphones. The Blackman gated signals, with a 2 ms rise/fall time and total signal duration of 6 msec, were delivered to the ear. Pass criteria included repeatable wave V at 30 dBnHL in both ears (Keogh and Beahan, 2009).

2.4.3.5 Bone conduction ABR.

Bone conduction ABR was performed using a Biologic auditory evoked potentials (v.6.2.0). Broadband clicks with alternating polarity were presented at a rate of 33.1/s through a Radioear B70A oscillator held on the temporal bone in a superoposterior auricular position (Yang and Stuart, 1990). Response determination was similar to click ABR. The criterion for normal bone conduction ABR was a repeatable ABR trace at an intensity of 15 dBnHL (Stuart et al, 1990; Cone-Wesson, 1995).

2.4.3.6 Auditory Steady State Response (ASSR).

ASSR evaluation was performed using the Multiple Auditory Steady State Response (MASTER; v.2.04.i00) from Biologic Systems (John and Picton, 2000).

Stimuli consisted of sine waves at carrier frequencies of 500, 1,000, 2,000 and 4,000 Hz with 100% amplitude modulation and 20% frequency modulation. Up to a level of 80 dB HL, all of the four frequencies were presented simultaneously to each ear. Once the stimulus level exceeded 80 dB HL, the instrument permitted presentation of only one frequency, one ear at a time, in order to avoid damage due to high intensity. Modulation frequencies were 82, 87, 91 and 96 Hz for the left ear and 84, 89, 94 and 99 Hz for the right ear (John and Picton, 2000). Air conducted stimuli were presented through insert ear phones while bone conducted stimuli were presented via a Radioear B-71 bone oscillator held in position with a headband fastened with Velcro. The signals were presented using 10 dB steps down to 30 dB HL for both air and bone conducted stimuli. Pass criteria for both air and bone conducted ASSR was a response at 30 dB HL from 500 to 4000 Hz with a significance level of $p < .05$.

2.4.3.7 Visual reinforcement audiometry (VRA).

For children older than 7 mo of age, behavioural assessment of hearing was performed with VRA in the sound field using an Interacoustics AC40 clinical audiometer. Testing was conducted in a sound-treated test booth with a single room set up. VRA thresholds or minimum response levels (MRLs) were determined for warble tones in the frequency range of 500 to 4,000 Hz presented through loudspeakers kept at 1 m distance and at an angle of 45° from the child's ears. A 10 dB step size (20 down, 10 up) was chosen to allow for quick convergence on VRA threshold or MRL (Widen et al, 2000). For the purpose of this study, normal hearing was defined as MRLs of 30 dB SPL at 500 Hz and 25 dB SPL from 1,000 to 4,000 Hz (Keogh and Beahan, 2009).

2.4.3.8 Bone conduction VRA.

Bone conduction VRA was performed when the sound field MRLs were above normal levels. Warble tones were presented at 1,000 to 4,000 Hz via a bone conductor placed on the mastoid. Normal bone conduction results were defined as MRLs of 15 dB from 100 to 4,000 Hz (Vander Werff et al, 2009).

2.4.4 Conductive hearing loss and middle ear pathology

Hearing sensitivity can vary widely in the presence of middle ear pathology. In the current study, the term “middle ear pathology” includes infants with middle ear

pathology with normal hearing and infants with conductive or mixed hearing loss. The following sections describe algorithms for determining middle ear pathology with normal hearing and conductive hearing loss.

2.4.4.1 Algorithm to determine middle ear pathology with normal hearing.

Hearing was considered to be functionally normal in the presence of middle ear dysfunction if (1) high frequency tympanogram showed flat-type tympanogram with no change in admittance or (2) 226 Hz tympanogram showed flat B-type tympanogram with no change in admittance along with (3) wave V present up to 30dBnHL with click ABR and/or (4) wave V present down to 30 dBnHL with 1,000 Hz tone burst ABR and/or (5) free field VRA thresholds of 30 dB SPL at 500 Hz and 25 dB SPL at 1,000 to 4,000 Hz and/or (6) response present down to 30 dB HL from 500 to 4,000 Hz with ASSR and (7) TEOAEs not present >6 dB signal-to-noise ratio (SNR) in at least three of four frequency bands.

2.4.4.2 Algorithm to determine middle ear pathology with conductive hearing loss.

Using results from the test battery, conductive hearing loss due to middle ear pathology was considered to be present if (1) high frequency tympanogram showed flat-type tympanogram with no recordable middle ear pressure or compliance, or (2) 226 Hz tympanogram showed flat B-type tympanogram with no change in admittance along with (3) TEOAEs not present at >6 dB SNR in at least three of four frequency bands including 1,500 and 4,000 Hz and (4) Wave V present above 30 dBnHL with prolonged or normal latencies with Click ABR and (5) Wave V present above 30 dBnHL with 1,000 Hz tone burst ABR and/or (6) VRA thresholds above 25 dB SPL in any of the frequencies between 1,000 and 4,000 Hz and above 30 dB SPL at 500 Hz and/or (7) responses above 30 dB HL in any of the frequencies between 500 and 4,000 Hz with ASSR and (8) normal bone conduction ABR/ASSR/VRA.

2.4.5 Classification of hearing loss

The Healthy Hearing program has adopted an operational definition of normal hearing to correspond to an ABR threshold of 30 dBnHL. While there are no standard methods of classifying the severity of hearing loss based on ABR thresholds, the present study adopted the conservative approach of the Healthy Hearing program in

classifying severity based on the average of click and 1000 Hz air conducted ABR thresholds. This procedure was similar to the classification of hearing thresholds based on pure tone evaluation as given by Jerger and Jerger (1980). Worse ear was used to calculate the degree of hearing loss. Average air conducted thresholds up to 40 dBnHL were classified as mild, 41-55 dB nHL as moderate, 56-70 dBnHL as moderately severe, 71-85 dBnHL as severe and above 85 dBnHL as profound (Jerger and Jerger, 1980).

2.5 RESULTS

2.5.1 Initial assessment outcomes

Of the 234 infants (140 males, 94 females) referred for diagnostic audiology assessment, 63 (26.9%) were ATSI and 171 (73.1%) were non-ATSI. A total of 211 infants (128 males, 83 females) attended the initial diagnostic audiology assessment at the Audiology department. Of these, 54 infants (25.6%) were ATSI and 157 (74.4%) were non-ATSI. The remaining 23 infants (12 males, 11 females) were either referred elsewhere or failed to attend the appointment. Of the 23 infants who did not attend the initial diagnostic appointment, 9 were ATSI and 14 were non-ATSI. The difference between the nonattendance rates of ATSI and non-ATSI infants (14.29% and 8.19% respectively) was not significant ($\chi^2 = 1.305$, $p > .05$).

The mean age of infants at the time of first diagnostic assessment was 47.5 days (SD = 31.30, range = 2-121 days). The mean time difference between screening and first diagnostic assessment was 24.4 days (SD = 22.69, range = 0-117 days). Delays in performing the initial diagnostic assessment were due to infants not being medically ready for audiological assessment, parents having difficulty travelling long distances with young infants and other children, family circumstances including the partner's availability to accompany mother and infant, or families missing earlier appointments.

Figure 2.2 shows the audiological outcomes for infants at the first appointment, as determined by the algorithm described earlier in "Methods". Of the 211 infants who attended the appointment, 99 (46.92%) had normal hearing with normal middle ear functioning in both ears. No gender difference was seen in these 99 infants ($\chi^2 = 0.691$, $p > .05$). Eighteen (8.53%) infants had middle ear pathology with

normal hearing, 47 (22.27%) had middle ear pathology with conductive hearing loss, 26 (12.32%) had sensorineural hearing loss and four (1.90%) had mixed hearing loss. Type of hearing loss could not be determined in 17 (8.06%) infants during the initial assessment.

2.5.1.1 Conductive hearing loss.

Prevalence of conductive hearing loss was 2.97 per 1000 (47 of 15,824 babies screened) while prevalence of sensorineural hearing loss was 1.64 per 1,000 (26 of 15, 824). Prevalence of conductive hearing loss was 1.8 times that of sensorineural hearing loss in the study cohort.

One infant had unilateral permanent conductive hearing loss with congenital atresia. Of the remaining 46 infants with conductive hearing loss with no congenital ear anomalies, 24 infants had unilateral and 22 bilateral conductive hearing loss. Twenty-four infants had a mild degree of loss (17 unilateral and 7 bilateral), 18 had a moderate degree (5 unilateral and 13 bilateral) and 4 had a moderately severe degree of loss (2 unilateral and 2 bilateral).

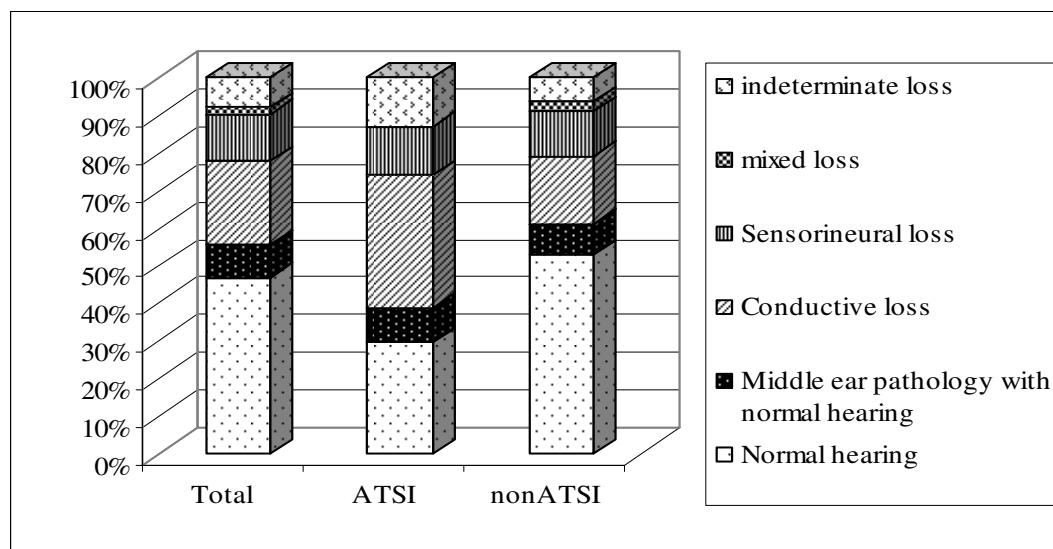


Figure 2.2: Audiological outcomes at the first diagnostic assessment for 211 infants (54 ATSI and 157 non-ATSI)

During the initial diagnostic assessment, 29.63% (16 of 54) of ATSI infants and 52.87% (83 of 157) of non-ATSI infants passed all the tests and had normal hearing with normal middle ear function in both ears. The difference between the two

groups was statistically significant ($\chi^2= 7.803$, $p=.0042$). The prevalence of conductive hearing loss was twice as high in ATSI infants (19 of 54, 35.19%) compared to non-ATSI infants (28 of 157, 17.83%). ATSI infants had a significantly higher prevalence of conductive hearing loss than non-ATSI infants ($\chi^2= 6.020$, $p=0.0141$). There was no significant difference between ATSI and non-ATSI infants in terms of prevalence of sensorineural hearing loss ($\chi^2=0.028$, $p>.05$), mixed hearing loss ($\chi^2=0.367$, $p>.05$) and indeterminate hearing loss ($\chi^2=1.552$, $p>.05$) (Table 2.1). There was no significant gender difference in rates of conductive loss between males (30 of 128, 23.44%) and females (17 of 83, 20.48%) ($\chi^2=0.112$, $p>.05$).

2.5.1.2 .Middle ear pathology.

As defined earlier, middle ear pathology included middle ear pathology with normal hearing and hearing loss. As shown in Figure 2.2, 18 infants had middle ear pathology with normal hearing, 47 infants had conductive hearing loss and four had mixed hearing loss during initial diagnostic assessment. Hence, a total of 69 infants out of 211 infants (29.5%) referred had middle ear pathology. The prevalence of middle ear pathology in neonates was 4.36 per 1,000 (69 of 15824) which was 2.7 times that of sensorineural hearing loss (1.64 of 1,000).

Table 2.1: Comparison of audiological outcomes of ATSI (n=54) and non-ATSI (n=157) infants at first diagnostic appointment

	Total	ATSI, n (%)	nonATSI, n (%)	z Value	Significance, p
Normal hearing	99	16 (29.63)	83 (52.87)	2.794	<.05
Middle ear pathology with normal hearing	18	5 (9.26)	13 (8.28)	-0.06	>.05
Conductive loss	47	19 (35.19)	28 (17.83)	2.455	<.05
Sensorineural loss	26	7 (12.96)	19 (12.10)	-0.074	>.05
mixed loss	4	0 (0)	4 (2.55)	0.607	>.05
indeterminate loss	17	7 (12.96)	10 (6.37)	1.245	>.05

Thirty-three infants had unilateral and 36 infants had bilateral middle ear pathology during initial assessment. There was no significant gender difference in the prevalence of middle ear pathology between males (45 of 128, 35.16%) and females (24 of 83, 28.92%) ($\chi^2=0.891$, $p>.05$). A total of 44.45% (24 of 54) of ATSI and 28.66% (45 of 171) of non-ATSI infants had middle ear pathology with normal hearing or with conductive or mixed hearing loss (Table 2.1). ATSI infants had significantly higher prevalence of middle ear pathology compared to non-ATSI infants ($\chi^2=3.859$, $p=.0495$).

2.5.2 Outcomes of review assessment

2.5.2.1 Outcomes for infants with conductive hearing loss

The mean time interval between the first and review assessments was 88.31 days (SD = 44.14, range = 23-182 days). Figure 2.3 shows the audiological outcomes of infants with conductive hearing loss at review assessment. Of the 47 infants with conductive hearing loss, one infant with congenital atresia was referred for ear, nose , and throat (ENT) consultation. The remaining 46 were scheduled for an audiology review in 6-8 wk. Among the infants scheduled for review, 19 were ATSI and 27 were non-ATSI. However, only 34 (34 of 46, 73.91%) infants (22 males, 12 females) attended the review. Of these 34 infants, 16 were ATSI and 18 were non-ATSI. Ten (three ATSI and seven non-ATSI infants) were either referred elsewhere or failed to attend the follow-up and a further two non-ATSI infants were not ready for testing at the time and, hence, were unavailable for review. Although the nonattendance rate appeared to be higher among the non-ATSI (9 of 27, 33.33%) than ATSI (3 of 19, 15.79%) infants, the difference was not statistically significant ($\chi^2=0.987$, $p>.05$).

During review, a total of 16 infants (47.06%, 11 males, five females) had normal hearing with normal middle ear function, 17 (50%, 11 males, six females) had conductive hearing loss and one female ATSI infant (2.94%) had an indeterminate finding. No significant gender effect was noted for infants with normal hearing ($\chi^2=0.011$, $p>.05$) or for those with conductive hearing loss ($\chi^2=0.000$, $p>.05$).

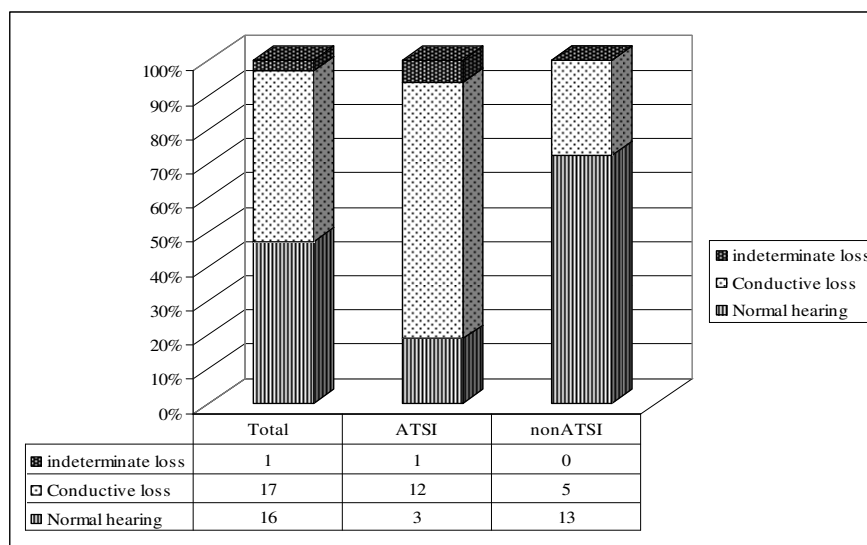


Figure 2.3: Review outcomes for ATSI (n=16) and non-ATSI (n=18) infants diagnosed with conductive hearing loss at first diagnostic assessment

Of the 17 infants with persistent conductive hearing loss, seven demonstrated unilateral and eight bilateral conductive hearing loss. Two infants had conductive hearing loss in the better ear as evaluated by VRA. Eight infants (four unilateral, two bilateral and two in the better ear) had a mild degree, seven (two unilateral and five bilateral) had a moderate and two (one unilateral and one bilateral) had a moderately severe degree of conductive hearing loss.

During review, 16 (three ATSI and 13 non-ATSI) infants had normal hearing with normal middle ear function in both ears. This accounted for 18.75% (3 of 16) of ATSI and 72.22% (13 of 18) of non-ATSI infants with normal hearing and this difference between the two groups of infants was statistically significant ($\chi^2=7.694$, $p=.0055$). Of the total 17 infants with conductive hearing loss, 12 were ATSI and five were non-ATSI. This accounted for 75% (12 of 16) of ATSI and 27.78% (5 of 18) of non-ATSI infants having persistent conductive hearing loss during review. This difference in prevalence of conductive hearing loss between the two groups of infants was statistically significant ($\chi^2=5.785$, $p=.0162$) (Table 2.2).

Table 2.2: Comparison of review outcomes of ATSI (n=16) and non-ATSI (n=18) infants diagnosed with conductive hearing loss at initial assessment

	Total	ATSI n (%)	nonATSI n (%)	z Value	Significance, p
Normal hearing	16	3 (18.75)	13 (72.22)	2.774	<.05
Conductive loss	17	12 (75)	5 (27.78)	2.405	<.05
indeterminate loss	1	1 (6.25)	0 (0)	0.06	>.05

ATSI = Aboriginal and Torres Strait Islander

Figure 2.4 shows comparison of outcomes of first assessment with review assessment. Sixteen infants (with conductive hearing loss at initial assessment) were found to have normal hearing during review, suggesting that conductive hearing loss (47.06%) infants. Unilateral conductive hearing loss had resolved in 10 infants and bilateral conductive loss in six infants. Seventeen of the 34 infants continued to show conductive hearing loss, suggesting that they had persistent hearing loss even after an average of 135 days.

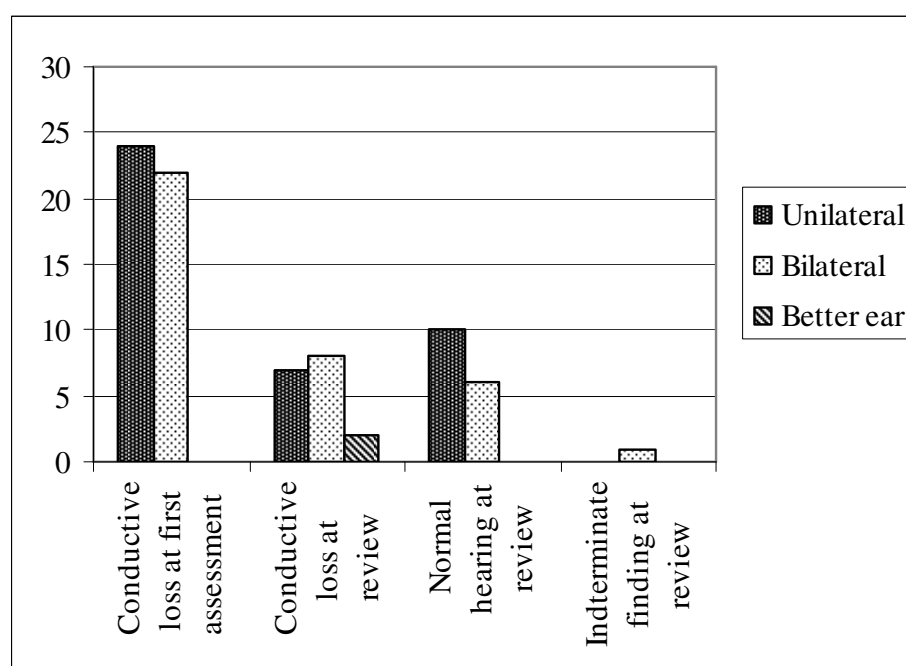


Figure 2.4: Audiological outcomes of infants with conductive hearing loss during initial and review assessments

The 17 infants (five non-ATSI and 12 ATSI) with conductive hearing loss constituted 8.06% (17 of 211) of the infants referred for diagnostic assessment. These

17 infants were referred for further management by ENT specialists and were considered for medical management including grommets, examination under anaesthesia, medication, and monitoring.

2.5.2.2 Outcomes for infants with middle ear pathology

Of the 64 infants with middle ear pathology scheduled for review, a total of 46 infants (30 males, 16 females) attended the review. Of the 46 infants, 18 were ATSI and 28 were non-ATSI. Nineteen infants had normal hearing with normal middle ear function, six had middle ear pathology with normal hearing and 17 had conductive hearing loss and hearing status could not be determined in four infants. Thus, a total of 23 infants had middle ear pathology during the review (10 infants unilateral, 13

bilateral). There was no gender difference in terms of middle ear pathology between males (17 of 30, 56.67%) and females (nine of 16, 56.25%) ($\chi^2=0.001$, $p>0.05$).

Of the 19 infants with normal hearing and normal middle ear functioning, four (four of 18, 22.22%) were ATSI and 15 (15 of 28, 53.57%) were non-ATSI (Figure 2.5). A significantly higher proportion of non-ATSI infants demonstrated normal hearing with normal middle ear functioning during review ($\chi^2=4.441$, $p= .0351$). Of the 23 infants with middle ear pathology, 13 (13 of 18, 72.22%) were ATSI and 10 (10 of 28, 35.71%) were non-ATSI. ATSI infants had a significantly higher rate of middle ear pathology at review than non-ATSI infants ($\chi^2=4.472$, $p= .0345$) (Table 2.3).

Table 2.3: Comparison of review outcomes of ATSI (n=18) and non-ATSI (n=28) infants diagnosed with middle ear pathology at initial assessment

	Total	ATSI, n (%)	nonATSI n (%)	zValue	Significance, p
Normal hearing	19	4 (22.22)	15 (53.57)	1.801	<.05
Middle ear pathology with normal hearing	6	1 (5.56)	5 (17.86)	0.76	>.05
Conductive loss	17	12 (66.67)	5 (17.86)	3.034	<.05
indeterminate loss	4	1 (5.56)	3 (10.71)	0.069	>.05

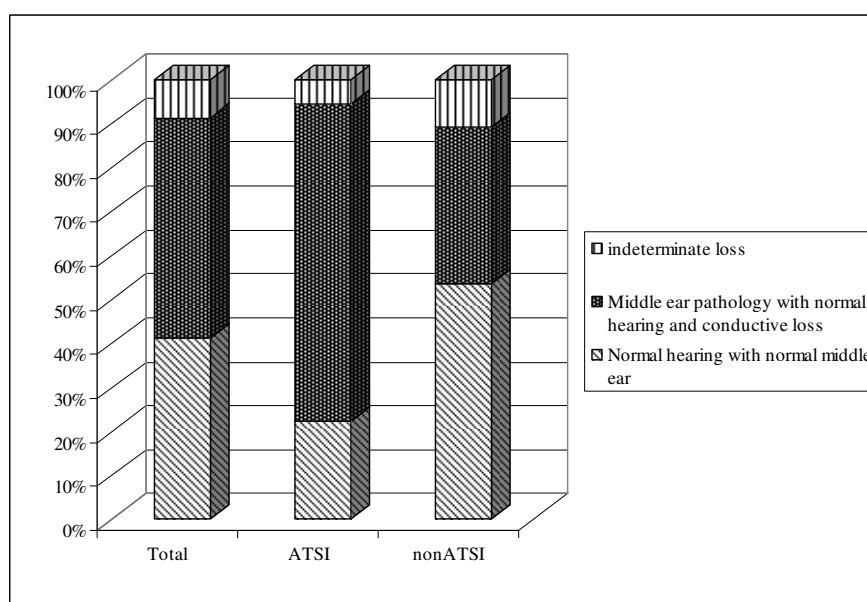


Figure 2.5: Review outcomes of ATSI (n=18) and non-ATSI (n=28) infants diagnosed with middle ear pathology at first diagnostic assessment

2.6 Discussion

One of the aims of the study was to evaluate the prevalence of conductive hearing loss and middle ear pathology in infants referred through the Healthy Hearing screening program in north Queensland. Using a battery of audiological tests, conductive hearing loss was found to be a common diagnosis among infants referred through screening. Prevalence of conductive hearing loss was found to be 2.97 per 1,000 during initial diagnostic assessment which was 1.8 times that for sensorineural hearing loss (1.64/1000). This agrees very well with findings of Boone et al (2005) who also found prevalence of conductive hearing loss to be 1.8 times that of sensorineural hearing loss in infants referred through an American newborn hearing screening program.

However, the reported figures vary for prevalence of conductive hearing loss in infants. White et al (1993) have reported that 17 of 1,000 well babies and 36 of 1,000 babies in newborn intensive care units had conductive hearing loss. Orlando and Prieve (1998) and Gorga et al (2001) reported that conductive hearing losses due to congenital middle ear effusion or more permanent external and middle ear problems occur at a rate 30 times greater than sensorineural hearing losses in young infants. The present study found that 22.27% of infants who were seen following failure of AABR hearing screening were diagnosed to have conductive hearing loss.

This variation in prevalence figures across studies could be attributed to differences in the screening methods used in the hearing screening programs. In the present study, the infants were screening using AABR, while the other studies cited have used either OAE or a combination of OAE and AABR. It is well known that external and middle ear abnormalities can have a significant effect on the OAE screening results, but not on AABR screening results (Northrop et al, 1999). Although the nature of middle ear pathology was not investigated using methods such as amniocentesis in any of these studies, it can be expected that the amniotic fluid retained in the middle ear cavity would be the most likely causative factor (Boone et al, 2005).

The second goal of the study was to compare the prevalence of conductive hearing loss and middle ear pathology between ATSI and non-ATSI infants. Nonattendance rates were not significantly different between ATSI and non-ATSI infants during both initial and review assessments, and hence, did not influence the results in any particular group. A significantly higher proportion of non-ATSI infants showed normal hearing with normal middle ear function at both initial and review assessment (Tables 2.1-2.3). ATSI infants had a higher prevalence of middle ear pathology and conductive hearing loss than non-ATSI infants both at initial diagnostic as well as at review assessments. During initial assessment, the prevalence of conductive hearing loss in ATSI infants (35.19%) was nearly twice that of non-ATSI infants (17.83%). During review, conductive hearing loss was found to persist in 75% of ATSI infants compared to 27.78% of non-ATSI infants. This indicates that ATSI infants demonstrated poorer resolution of conductive hearing loss than non-ATSI infants. Thus, the middle ear function of ATSI infants appears to differ from non-ATSI infants from early infancy. These findings are consistent with the earlier reports of high rates of middle ear pathology in ATSI infants. For example, Boswell and Nienhuys (1995) found that 95% of 22 6- to 8- wk-old Aboriginal infants had otitis media with effusion compared to 30% among non-Aboriginal infants. In a cross-sectional study of Aboriginal infants, Foreman (1987) found the prevalence of perforation was 11% at age of 0-6 mo and 43% at 7-12 mo. However, longitudinal studies that document the natural history of MEE and conductive hearing loss in young ATSI infants are very scarce. In the first longitudinal audiological and otoscopic study of 17 infants in the first year of life, Boswell and Nienhuys (1996) reported that Aboriginal infants had middle ear aeration and normal hearing within 2

wk of birth. During follow-up over a period of 12 mo, 90% of non-Aboriginal infants and 0% of Aboriginal infants had normal middle ears during repeated observations. Longitudinal microbiological studies have shown early bacterial colonisation with multiple bacterial types resulting in prolonged carriage and Eustachian tube damage to be the main reason for persistent otitis media in Aboriginal children (Leach et al, 1994; Morris et al, 2009). Other contributing factors include overcrowding, poor hygiene, and lowered resistance from nutritional and genetic causes (Scrimshaw et al, 1968; Canty et al, 1975; Kavanagh, 1986; Leach et al, 1994; Runcie and Bailie, 2000; Leach and Morris, 2001; Smith-Vaughan et al, 2001; Morris et al, 2009; Jacoby et al, 2008). However, there are no longitudinal studies that link these environmental factors to early onset and persistent otitis media from early infancy in these children. There is, thus, a need for well controlled longitudinal studies to document the natural history of MEE along with hearing status in Australian Aboriginal infants.

There is also limited longitudinal research to determine the course of neonatal MEE and its relation to chronic MEE in the general population. In humans, although the resorption of mesenchyme from the middle ear cleft is reported to be complete by the eighth foetal month, it can vary up to the thirteenth postnatal month (Guggenheim et al, 1956; Buch and Jorgensen, 1964; Arey, 1968) with some children retaining large amounts of mesenchyme until the onset of puberty (Takahara et al, 1986). Temporal bone studies have shown that persistent amniotic fluid cellular content can spread to various middle ear compartments and cause extensive histopathological changes due to foreign body giant cell reaction, the severity of which is related to the amount of amniotic cellular content (Piza et al, 1989; Northrop et al, 1999; Palva, Northrop & Ramsay, 1999). There was also a linear relationship between increasing age and organised reaction of the middle ear mucosa with the spectrum of middle ear pathology ranging from minimal to formation of reactive polyps and extensive fibrosis (Bacsik, 1977; Palva et al, 2001). Massive granulation tissue with numerous pseudocysts and secretions trapped to many attic compartments, together with the hyperplastic mucosa with secretory elements are postulated to lower the resistance of the middle ear to viral or bacterial infection, thereby predisposing these infants with persistent effusion to otitis media (Northrop et al, 1999; Ramsay et al, 2001).

A handful of studies have found that newborns with persistent MEE were more likely to develop chronic MEE. Doyle et al (2004) found that 58% of 14 infants with persistent neonatal MEE at 30-48 hr of life developed chronic otitis media with effusion during the first year of life compared to 20% of 15 infants who had no MEE in the first 2 days of life. In a study of 238 infants, Pereira, Azevedo, and Testa (2010) found that 57% of 16 infants who failed their hearing screening in the first month of life due to conductive pathology had otitis media in the first year of life compared to 23% of 9 infants who passed their hearing screening. Boswell et al (1993) noted that once middle ear disease started within the first 8 wk of life and was established in Australian Aboriginal infants, it was persistent despite treatment. It remains to be seen whether this persistent MEE and resulting damage to middle ear tissues in young infants could be predisposing their ear for further infections, thereby rendering them “otitis prone”. There are currently no well-defined, longitudinal studies that have monitored the resolution of middle ear fluid from birth to the first few months of life. Further longitudinal studies are needed to study the natural history and resolution of MEE with or without hearing loss in early infancy in both ATSI and non-ATSI infants to determine if MEE is a precursor for later persistent middle ear infections.

The third aim of the present study was to review the outcomes of the infants diagnosed with middle ear pathology. The results showed that middle ear pathology had resolved in 41.30% (19 of 46) of infants seen at review. This figure is much lower than the 65% resolution of MEE reported by Boone et al (2005). However, this difference could be due to results not being available for 18 infants due to loss to follow up, transfer, incomplete results or infants not ready for review.

The fact that 17 infants had persistent conductive hearing loss even at an average age of 135 days suggests that middle ear pathology is likely to persist beyond the first few weeks of life. This is consistent with temporal bone studies that have reported presence of mesenchyme in the middle ear cleft up to 13 mo of age (Guggenheim et al, 1956; Kasemsuwan et al 1996; Jaisinghani et al, 1999). However, these results need to be substantiated using audiological and radiological studies in live infants. While the natural history of treated or untreated MEE in older infants and

children is well documented (Rosenfeld et al, 2004), there are no longitudinal studies that have investigated the resolution of MEE in children in the first 12 mo of life.

Of the 23 infants with middle ear pathology during review, 17 with conductive hearing loss were referred for medical management including grommets insertion, medication and follow up appointments. The remaining six infants were advised audiological review to monitor their middle ear status. These management strategies are in line with the most commonly recommended treatments for MEE in older children including watchful waiting for a certain amount of time, oral antibiotics and surgical drainage with or without placement of ventilation tubes (Rosenfeld et al, 2004; Boone et al, 2005).

A number of factors could have affected the results of the present study. First, the study only assessed those infants who failed the AABR screen. It is possible that some infants with middle ear pathology with or without slight to mild hearing loss could have passed the screening. Unlike OAE screening, external and middle ear abnormalities do not have a significant effect on the results of AABR screening (El-Refaie et al, 1996; Northrop et al, 1999). Second, a number of infants were lost to follow up in both the initial and review assessment sessions. Hence the prevalence for middle ear pathology and conductive hearing loss could have been under- or over-estimated. Third, the present study did not consider the review outcomes of the infants diagnosed with mixed hearing loss or indeterminate hearing loss during the initial assessment. Inclusion of these infants may have influenced the prevalence rates of conductive hearing loss and middle ear pathology in the present study.

Although the main aim of neonatal hearing screening programs is the identification of congenital permanent hearing loss, the presence of middle ear pathology can prolong the diagnosis of permanent hearing loss. Hence, an accurate and non-invasive tool is needed at the time of screening to distinguish middle ear pathology from sensorineural hearing losses. Not only may this streamline the management strategies for the respective types of hearing loss, but it may also facilitate prioritisation of infants for follow-up appointments and reduce parental anxiety. To this end, future research with multi-frequency tympanometry, sweep

frequency tympanometry, acoustic reflex or wide band reflectance used as an adjunct to the AABR screening tool would be beneficial.

In summary, middle ear pathology with or without conductive hearing loss was found to be a common cause of referral from the newborn hearing screening program in north Queensland. The prevalence of conductive hearing loss was 2.97 per 1,000 while prevalence of middle ear pathology was 4.36 per 1,000. Australian indigenous infants had higher prevalence rates of conductive hearing loss and middle ear pathology than their non-indigenous counterparts. Indigenous infants also showed poor resolution of middle ear pathology with time. The present study found that 8.06% of the referred infants had persistent conductive hearing loss exceeding 3 mo and required medical management for their condition including follow up appointments, antibiotic treatment and grommet insertion. Future research utilising a direct measure of middle ear function as an adjunct to the AABR screening tool to distinguish between middle ear pathology from sensorineural hearing loss may facilitate prioritisation of infants for assessment, reduce parental anxiety and streamline the management strategies for respective types of hearing loss.

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Chapter Three: Normative Wideband Absorbance Measures In Healthy Neonates

3.1 Background

Normative data on WBA has been developed in neonates using DPOAE, a screening tool, as a reference standard for middle ear status (Sanford et al., 2009; Hunter et al., 2010). For ethical reasons, myringotomy is not justified to determine middle ear status in asymptomatic neonates. Passing DPOAE is not an ideal reference standard because DPOAE can be present in some ears with middle ear dysfunction. In the absence of a single gold standard to assess middle ear dysfunction in neonates, Mazlan and Kei (2012) recommended the use of a battery of tests to determine middle ear status in this population. A battery of tests may provide a robust measure of middle ear function without resorting to invasive procedures such as myringotomy.

Normative data for WBA was developed in neonates who passed a battery of tests that included automated auditory brain response, high frequency tympanometry, acoustic stapedial reflex, transient evoked otoacoustic emissions and DPOAE. The normative data developed in the present study and comparison of the results with other normative WBA studies in neonates are presented in Chapter Three of this thesis.

Chapter Three of this thesis, entitled, “Normative wideband absorbance measures in healthy neonates”, is based on the article published in *International Journal of Paediatric Otorhinolaryngology*. This published article is inserted into this thesis with some modifications. For example, the results of the study are presented in terms of absorbance rather than reflectance. This modification is necessary to ensure consistency of the measure (absorbance) throughout the thesis. Additionally, the formatting of section sub-headings has been modified from the original publication to match the thesis format. The referencing format of the article is retained as per the *International Journal of Paediatric Otorhinolaryngology* journal format.

Aithal, S., Kei, J., Driscoll, C., & Khan, A. (2013). Normative wideband reflectance measures in healthy neonates. *International Journal of Paediatric Otolaryngology* 77: 29-35.

3.2 Abstract

Objective: Presently, normative wideband reflectance data are available for neonates who have passed a distortion product otoacoustic emission test. However, passing the distortion product otoacoustic emission test alone does not ensure normal middle ear function. The objective of this study was to establish normative wideband reflectance data in healthy neonates with normal middle ear function, as justified by passing a battery of tests.

Method: Wideband reflectance was measured in 66 infants (mean age = 46.0 h, SD = 21.0, range = 13.3–116.5 h) who passed a test battery that included high frequency (1000 Hz) tympanometry, acoustic stapedial reflex, transient evoked otoacoustic emissions and distortion product otoacoustic emissions.

Results: The analysis of variance (ANOVA) results showed significant variations of reflectance across the frequencies. There was no significant difference between ears and genders. The median reflectance reached a minimum of 0.21–0.24 at 1–2 kHz, but increased to 0.45–0.59 below 1 kHz and 0.24–0.52 above 2 kHz.

Conclusions: The normative reflectance data established in the present study were in agreement with, but marginally smaller than, those of previous normative studies, except for the Keefe et al. (2000) study. While the use of a test battery approach to ensure normal middle ear function in neonates has resulted in slightly reduced reflectance across most frequencies when compared to studies that have used only otoacoustic emissions, further research is needed to accurately determine the middle ear status of neonates using test performance measures.

Keywords wideband absorbance, neonates; normative.

3.3 Introduction

Determination of middle ear function is an important aspect of diagnostic assessment in infants and young children. The standard tools used to determine the middle ear status in older children are neither efficient nor accurate in evaluating neonates. Myringotomy, which is the gold standard to determine middle ear fluid, is

neither ethical nor justified with neonates in a screening context. Both otoscopy and pneumatic otoscopy are not effective in neonates due to difficulties in visualising the tympanic membrane [1-4]. Conventional 226 Hz tympanometry has been found to be inaccurate in assessing infants younger than six months of age due to differences in acoustical and anatomical properties between adults and young infants [5-11]. Several studies have recommended high frequency (1000 Hz) tympanometry (HFT) for the assessment of middle ear function in infants less than six months of age [6, 10-12]. Nonetheless, recent studies [13, 14] that have compared the test performance of HFT and wideband absorbance (WBA) with distortion product otoacoustic emissions (DPOAE) in newborns found that the WBA test predicted DPOAE outcomes more accurately than the HFT.

The WBA test measures the power absorbance at ambient pressure using a wideband stimulus such as a click or chirp which covers a frequency range from 0.2 to 8 kHz. Power absorbance (1-power reflectance) is defined as the ratio of absorbed energy to incident energy [15] and ranges from 1 (representing total absorbance of sound into the middle ear) to 0 (representing complete reflectance of sound by the tympanic membrane). Several studies have shown that, at all ages, power absorbance is the lowest at frequencies below 1kHz and above 4 kHz and highest in the frequency region between 1 and 4 kHz, which corresponds to the most effective frequency region of the middle ear transfer function [13, 16-18].

The WBA test has been shown to be useful in the assessment of middle ear function in neonates [13-14, 19-23] and is, therefore, recommended as an adjunct tool with newborn hearing screening programs. For instance, Keefe et al. [20] demonstrated that inclusion of the WBA test in newborn hearing screening programs decreased the false positive rates from 5% to 1%, thus, suggesting that information on middle ear status improves the ability to predict hearing status. Sanford et al. [13] measured WBA in 455 healthy, full-term newborn infant ears that passed their DPOAE screening within 24 h of birth and compared the findings with 80 ears that were referred. They found that the referred ears had lower energy absorbance between 0.4 and 2.5 kHz when compared with those that passed the DPOAE test. In a study of 127 infants in screening and diagnostic test conditions, Vander Werff et al. [22] also

found that infants who failed DPOAE screening had lower absorbance in the range from 0.63 to 2 kHz than infants who passed the screening.

Despite its potential regarding the assessment of middle ear function in infants, there are limited normative WBA data for this population. Keefe et al. [24] reported the first set of normative WBA data in 2081 neonates. However, this study did not include any measure to determine the middle ear status of the neonates. It is only in recent years that studies have reported WBA results in healthy neonates with normal middle ear function as determined by a pass result in either DPOAE [13, 14, 21, 22] or transient evoked otoacoustic emissions (TEOAE) testing [25]. In a study of 324 newborns, Hunter et al. [14] described normative WBA regions for predicting DPOAE status in newborns. Hunter et al. [14] also proposed the use of absorbance area indices wherein the absorbance values are averaged over a specified frequency range (e.g., 1-2 kHz, 1-4 kHz, and 2-6 kHz). They found that area indices at 2 kHz or involving 2 kHz, successfully differentiated between ears that passed or referred on the DPOAE test, with areas under the ROC curve of 0.93 and 0.90, respectively. Merchant et al. [21] described normative absorbance and transmittance measurements on 12 ears from seven newborns that passed DPOAE screening. They found that the mean power absorbance was minimum (0.4) at 500 Hz and increased with frequency until 2 kHz where it reached a maximum of 0.82 and then decreased with frequency above 2 kHz.

As can be seen from the above studies, the DPOAE is often used as the reference standard to determine normal middle ear function in infants. However, the DPOAE alone may not accurately identify minor or sub-clinical middle ear pathologies [26] and, hence, may not serve as an ideal reference standard [13, 14]. Similarly, other tests such as HFT, acoustic stapedial reflex (ASR) or TEOAE in isolation are not effective measures of middle ear function in neonates. For example, there are unresolved issues for interpreting HFT results. To date, there are no universally agreed methods for interpreting HFT results, nor is there agreement regarding the test parameter or combination of parameters that are most sensitive to middle ear dysfunction in neonates [27]. Although normative ASR data is now available for neonates [28, 29], the ASR test alone cannot determine middle ear status as the presence or absence of ASR is dependent on several factors such as hearing

sensitivity and auditory function up to the brainstem region. Finally, TEOAEs alone are not perfect as a reference standard because TEOAEs can be recorded in some ears with middle ear dysfunction [30, 31].

In the absence of a single test for accurately identifying middle ear dysfunction in neonates, Mazlan and Kei [32] suggested that the use of a battery of tests including TEOAE, HFT and ASR tests may be an accurate measure for detecting middle ear dysfunction in young infants. Whilst a single measure such as HFT or DPOAE may not be sensitive to subtle middle ear dysfunction, a battery of tests may provide a robust measure of middle ear function for the evaluation of WBA in neonates. Such a battery may also provide the best reference standard available for newborns without resorting to invasive procedures such as myringotomy. To date, there are no WBA studies that have used a combination of tests as a reference standard to determine middle ear status in healthy neonates. The objective of this study was to describe normative WBA measures in healthy neonates who passed all tests in a reference standard battery that included AABR, HFT, ASR, TEOAE and DPOAE tests.

3.4 Method

3.4.1 Participants

A total of 195 (107 males, 88 females) healthy neonates were enrolled in the study. Only 66 neonates (35 males, 31 females) who passed all tests in the test battery in one or both ears were selected for the study. A total of 23 neonates passed the test battery in the right ear only, 21 passed in the left ear only and 22 neonates passed in both ears. When a neonate passed the test battery in both ears, either the right or left ear was chosen randomly for inclusion in the data analysis. Altogether, 66 ears (32 right and 34 left ears) that passed all the tests in the test protocol were included in the study.

Mean gestational age of the neonates was 38.7 weeks (SD = 5.01, range = 36-42 weeks). Thirty-four neonates (51.5%) were born via spontaneous vaginal delivery, six (9.1%) via assisted vaginal delivery, 24 (36.4%) via caesarean delivery and information was not available for two neonates (3%). Mean birth weight was 3534.9 g

(SD= 468.7, Range = 2290-4640 g). Fifty-five neonates (83.3%) were breast-fed, five (7.6%) were bottle-fed and information was not available for six (9.1%) neonates. Mean age of the neonates at the time of testing was 46.0 h (SD= 20.9, Range = 13.3-116.5 h). Fifty-five neonates (83.3%) were asleep during testing, while four (6.1%) were awake but quiet, three (4.5%) were awake and restless, one (1.5%) was being fed and information was not available for three (4.5%) neonates.

3.4.2 Test battery

Tympanometry was performed using a GN Otometrics Otofex acoustic immittance device with a 1000 Hz probe tone. During the test, the admittance (Y) was measured as the pressure was changed from +200 to -400 daPa at a rate of 400 daPa/s. A visual classification system was used to classify the tympanometric results. The pass criterion was a single positively peaked tympanogram with middle ear pressure between 50 and -150 daPa [33-35].

ASR was measured using the same Otofex instrument. ASR responses were recorded at the tympanometric peak pressure for a 2 kHz tone presented ipsilaterally in the presence of a 1000Hz probe tone. The stimulus tone was presented for 1 s at an intensity level starting at 70 dB HL using a manual threshold search mode. A change in admittance exceeding 0.04 mmho was considered as an ASR response [29]. A pass was awarded if an ASR was present up to 90 dB HL [28].

TEOAE was performed using a Biologic Navigator Plus. The signal consisted of wide band clicks of 80 μ s duration, at a target amplitude of 80dBpkSPL. The pass criteria included reproducibility of at least 70% and a difference between the amplitude of the emissions and the associated noise floor of at least 3 dB at 2, 3 and 4 kHz [22, 34].

DPOAE was performed using the same Biologic device. DPOAEs were measured in response to pairs of primary tones with F2 set at 1.5, 2, 3, 4, 6 and 8 kHz. The F2/F1 ratio was 1.2 for each primary pair. The level of F1 was 65 dBSPL and F2 was 55 dBSPL. Pass criteria included if the noise level was less than 0 dBSPL and the difference between the amplitude of the emission and associated noise floor was at least 6 dB in at least three out of four frequencies from 2 to 6 kHz [13, 14].

WBA was performed using a prototype research system developed by Interacoustics. The Reflwin computerised system consisted of a Windows-based computer, a 24 bit resolution sound card, a pressure pump and controller system contained in an acoustic immittance instrument (AT235), and custom software for stimulus generation and data acquisition. Calibration was performed every day [36] to determine the source reflectance and incident sound pressure associated with the probe and its transducers based on acoustic measurements in four rigid walled cylindrical calibration tubes.

Ambient WBA measurements were obtained by recording acoustic response to clicks, presented at 55 dB SPL and at a rate of one click per 46 ms, to a neonate's ear. Responses from a total of 16 clicks were averaged for each measurement and reflectance was calculated for each response. The WBA response consisted of 16 data points (at 1/3 octave frequencies from 0.25 to 8 kHz). A visual display with high absorbance at frequencies below 1 kHz served as an on-screen prompt that alerted the tester if there was a probe leak. A visual prompt also alerted the tester if the noise level was high. The data acquisition was very quick and the typical test time was less than 10 s.

3.4.3 Procedure

Ethical approval for the study was obtained from the Townsville Health Service District Institutional Ethics Committee and the University of Queensland Behavioural and Social Science Ethical Review Committee. Parents of healthy neonates at the maternity ward of Townsville Hospital were informed of the study by the nurses prior to the hearing screening of neonates. A consent form, approved by the Institutional ethics committees, was used to obtain written parental permission.

All measurements were performed in a quiet room in the well baby nursery. Hearing screening using AABR was always performed first by trained nursing staff. This was necessary so as not to interfere with the state-wide universal newborn hearing screening and to ensure functionally normal hearing. The AABR screening was done using the ALGO3 with a presentation level of 35 dBnHL. All the neonates included in the study passed the AABR screen in both ears. Following the AABR

screen, an audiologist performed the HFT, ASR, TEOAE and DPOAE tests, in no particular order and with the most accessible ear first. Only the ears that passed all the four tests were considered for inclusion in the study. The neonates were usually seen after feeding while in natural sleep or in an awake but quiet state. In a sleeping or cooperative neonate, the entire testing took an average of 20 min for both ears. In a wakeful or unsettled neonate, the testing time was 30 to 45 min depending on the activity state.

3.5 Results

A mixed model of analysis of variance (ANOVA) was applied to the data to evaluate the effects of gender, ear and frequency on absorbance. In this model, both gender and ear served as between-subject factors, and frequency (16 levels) as a within-subject factor. The Greenhouse and Geisser (G-G) approach [37] was used to compensate for the violation of compound symmetry and sphericity. The ANOVA results, shown in Table 3.1, revealed that there was a significant main effect for frequency [$F(78, 242) = 78.336$, $p < 0.001$]. The effects of ear, gender and their interactions with frequency were not significant ($p > 0.05$). To further investigate the frequency effect, multiple pairwise comparison tests with Bonferroni adjustment were performed on the absorbance data. Significant differences were found in 85 out of 120 paired comparisons (Table 3.2), showing large variations in absorbance across the frequencies. The absorbance was significantly different between 0.25-0.8 kHz and 1-2.5 kHz regions, 0.25-0.8 kHz and 5-8 kHz regions, and 1-2.5 kHz and 3-4 kHz regions. The absorbance did not vary significantly between 0.25-0.8 kHz and 3-4 kHz and between 1-2.5 kHz and 6-8 kHz.

Table 3.1: ANOVA results for wideband absorbance obtained from 66 neonates.

	F value	df	P value	Observed Power
Frequency	78.336	78, 242	0.000	1.00
Ear	0.001	1, 62	0.980	0.05
Gender	2.176	1, 62	0.145	0.31
Ear \times Gender	0.003	1, 62	0.959	0.05
Frequency \times Ear	0.269	4, 242	0.894	0.11
Frequency \times Gender	1.428	4, 242	0.236	0.44
Frequency \times Ear \times Gender	1.055	4, 242	0.379	0.33

In view of the insignificant ear and gender effects, the absorbance data were pooled across the ears and genders. Adjacent frequencies that did not differ significantly from each other were averaged to obtain absorbance area indices (AAIs). Table 3.3 shows the absorbance values for the 0, 5th, 10th, 50th, 90th, 95th and 100th percentiles at the individual frequencies and various AAIs for the neonates in the present study. The normative range for absorbance was defined as the region between the 10th and 90th percentiles [14]. Figure 3.1 shows the trend of the median and normative range of absorbance for individual frequencies. This trend reveals two minima of median absorbance of 0.41 and 0.48 at 0.5 and 4 kHz, respectively. The median absorbance attained maximum value of 0.79 at 1.5 kHz and 0.76 at 6 kHz.

The inter-quartile range (between the 25th and 75th percentiles) and the normative range (between the 10th and 90th percentiles) of absorbance are given in Table 3.4. The inter-quartile ranges of the absorbance at 2 kHz and above were generally lower than those at frequencies below 2 kHz. The inter-quartile range varied from 0.88 at 0.5 kHz to 0.51 at 8 kHz. The normative range varied from 0.82 at 0.3 kHz to 0.31 at 8 kHz. A general trend of decreasing normative range of absorbance with frequency was observed.

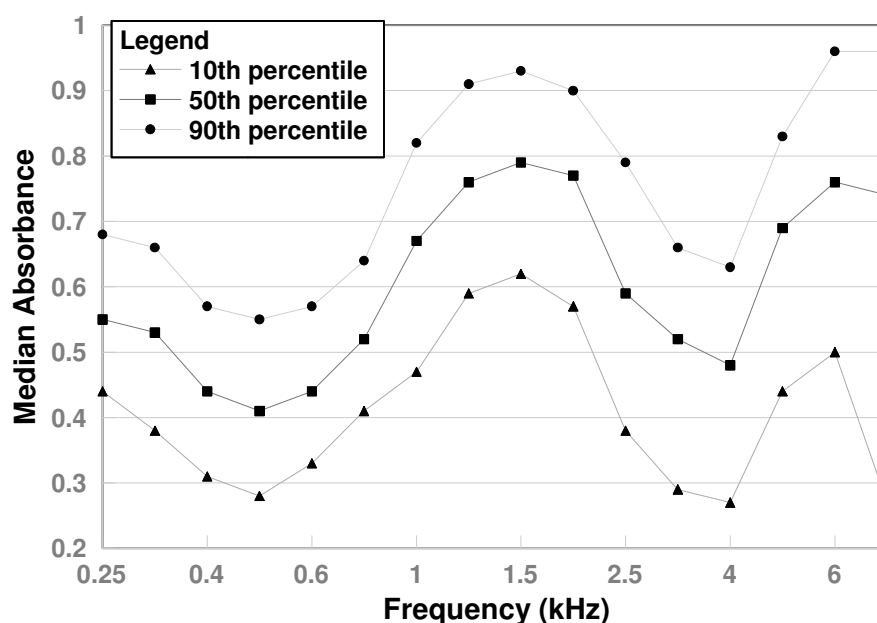


Figure 3.1: Median and normative range of absorbance for 66 neonates.

Table 3.2: Significance of difference between various absorbance area indices (Bonferroni correction applied)

Frequ- uency (kHz)	Frequency (kHz)														
	0.3	0.4	0.5	0.6	0.8	1	1.25	1.5	2	2.5	3	4	5	6	8
0.25	0.603	0.000	0.000	0.000	ns	0.000	0.000	0.000	0.000	ns	ns	0.013	0.000	0.000	Ns
0.3		0.000	0.000	0.000	ns	0.000	0.000	0.000	0.000	ns	ns	ns	0.000	0.000	0.005
0.4			0.000	ns	0.000	0.000	0.000	0.000	0.000	0.000	ns	ns	0.000	0.000	0.000
0.5				0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.000	ns	0.000	0.000	0.000
0.6					0.000	0.000	0.000	0.000	0.000	0.00	ns	ns	0.000	0.000	0.000
0.8						0.000	0.000	0.000	0.000	ns	ns	ns	0.000	0.000	0.005
1							0.000	0.000	ns	ns	0.000	0.000	Ns	Ns	ns
1.25								ns	ns	0.000	0.000	0.000	0.000	Ns	ns
1.5									0.014	0.000	0.000	0.000	0.000	Ns	ns
2										0.000	0.000	0.000	0.015	Ns	ns
2.5											0.000	0.000	ns	0.000	ns
3												ns	0.000	0.000	0.000
4													0.000	0.000	0.000
5														0.004	Ns
6															Ns

Figure 3.2 shows the variation of median absorbance with frequency for three earlier studies, in comparison to the present study. The findings of the present study are in agreement with those of the Shahnaz [25] and Hunter et al's [14] studies. However, the absorbance in the 1-4 kHz region in the present study was larger than those reported by Shahnaz [25] and Hunter et al. [14]. In comparison, the absorbance values reported by Keefe et al. [24] were larger than those obtained in the present study.

Table 3.3: Absorbance and absorbance area index (AAI) values for various percentiles (0, 5, 10, 25, 50, 75, 90, 95 and 100) for the infants with normal middle ear function as determined by a pass in all tests including HFT, ASR, TEOAE and DPOAE

Frequency (kHz)	Percentiles								
	0	5	10	25	50	75	90	95	100
0.25	0.20	0.29	0.44	0.48	0.55	0.63	0.68	0.72	0.76
0.31	0.33	0.35	0.38	0.45	0.53	0.58	0.66	0.70	0.75
0.40	0.22	0.25	0.31	0.37	0.44	0.51	0.57	0.64	0.69
0.5	0.15	0.23	0.28	0.34	0.41	0.46	0.55	0.58	0.63
0.63	0.18	0.29	0.33	0.38	0.44	0.51	0.57	0.61	0.62
0.80	0.22	0.32	0.41	0.44	0.52	0.58	0.64	0.70	0.73
1	0.26	0.39	0.47	0.56	0.67	0.75	0.82	0.89	0.93
1.25	0.31	0.48	0.59	0.68	0.76	0.85	0.91	0.94	0.96
1.50	0.50	0.56	0.62	0.69	0.79	0.87	0.93	0.94	0.98
2	0.49	0.51	0.57	0.63	0.77	0.85	0.90	0.93	0.99
2.5	0.25	0.32	0.38	0.47	0.59	0.69	0.79	0.86	0.89
3	0.23	0.25	0.29	0.39	0.52	0.60	0.66	0.77	0.86
4	0.08	0.19	0.27	0.36	0.48	0.59	0.63	0.67	0.83
5	0.03	0.36	0.44	0.57	0.69	0.77	0.83	0.88	0.94
6	0.31	0.43	0.50	0.66	0.76	0.90	0.96	0.97	0.99
8	0.10	0.25	0.27	0.42	0.74	0.91	0.96	0.98	0.99
.25-.31	0.29	0.33	0.40	0.47	0.54	0.60	0.66	0.69	0.75
.4-.8	0.19	0.28	0.34	0.41	0.45	0.50	0.59	0.61	0.62
1	0.26	0.39	0.47	0.56	0.67	0.75	0.82	0.89	0.93
1.25-2	0.49	0.57	0.62	0.70	0.77	0.84	0.87	0.90	0.94
2.5	0.25	0.32	0.38	0.47	0.59	0.69	0.79	0.86	0.89
3-4	0.17	0.24	0.29	0.39	0.50	0.60	0.63	0.70	0.85
5-8	0.30	0.42	0.47	0.60	0.72	0.82	0.85	0.90	0.92

Table 3.5 compares the absorbance for various AAI of the present study with the Hunter et al. [14] study. While the results between the two studies are comparable,

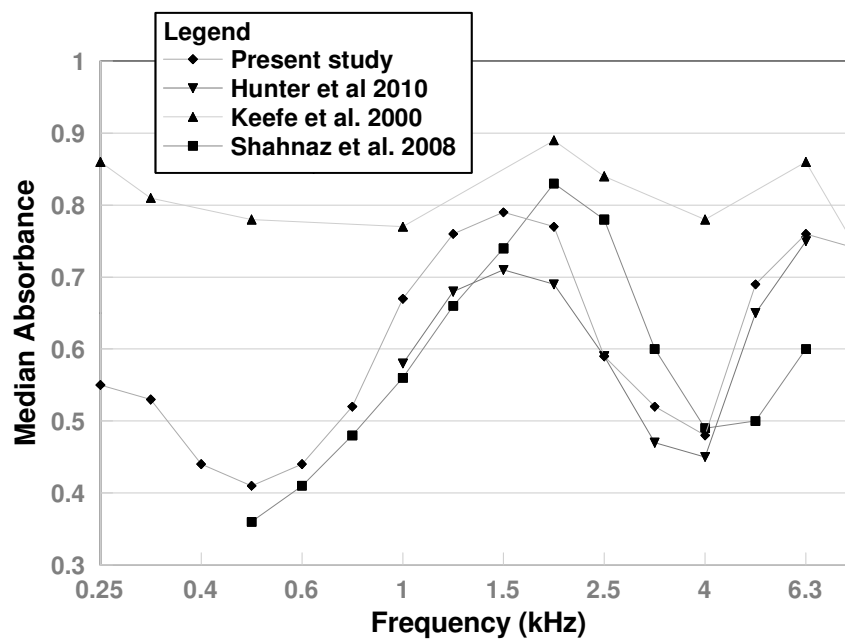
the absorbance values of the present study were generally larger than those of the Hunter et al. study.

Table 3.4: Inter-quartile range and normative range (between 10th and 90th percentiles) of absorbance at various individual frequencies and AAI's

Frequency (kHz)	Inter-quartile range	Normative range
0.25	0.16	0.25
0.30	0.13	0.28
0.40	0.14	0.27
0.5	0.12	0.27
0.60	0.12	0.23
0.80	0.14	0.23
1	0.19	0.36
1.25	0.17	0.32
1.50	0.18	0.31
2	0.22	0.33
2.5	0.22	0.41
3	0.21	0.37
4	0.23	0.36
5	0.20	0.39
6	0.24	0.46
8	0.49	0.69
.25-.31	0.13	0.26
.4-.8	0.09	0.25
1.00	0.19	0.36
1.25-2	0.14	0.25
2.50	0.22	0.41
3-4	0.20	0.34
5-8	0.22	0.39

Table 3.5: Comparison of absorbance results between the present and Hunter et al.'s (2010) studies for various AAI.

Frequency (Hz)	0th Percentile		10th percentile		90th Percentile		100th percentile	
	Hunter etal. 2010	Present study	Hunter etal. 2010	Present study	Hunter etal. 2010	Present study	Hunter etal. 2010	Present study
0.2-6 K	15.5	25.1	40.1	41.7	71.2	74.1	83.1	83.6
1-2K	17.7	28.9	47	56	82.8	89.2	93.2	96.2
1-4K	15.6	31.3	40.7	45.3	72.3	80.7	87	91.9
1-6K	13.9	27.3	40.3	45.7	74.7	82.6	85.1	92.8
2-4K	12.5	26.4	34.9	37.5	71	74.5	86.2	89.3
2-6K	11.5	23.2	35.8	40.6	74.6	79.4	88.5	91.5
4-6K	4.8	14.1	34.9	40.1	81.8	80.3	92.8	91.8
2K	15.5	49.3	44	56.9	89.9	90.2	98.5	98.6



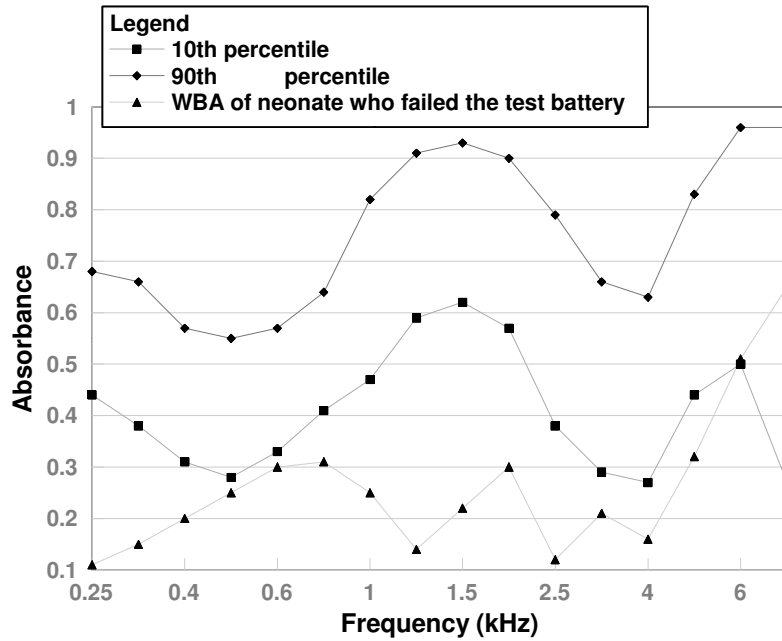


Figure 3.4: WBA from left ear of a 42-hour-old female neonate who failed the test battery

3.6 Discussion

The present study provided normative WBA measures in healthy neonates who passed the HFT, ASR, TEOAE and DPOAE tests. WBA measures were obtained for individual frequencies from 0.25 to 8 kHz as well as AAI's that were averaged over different frequency regions (Table 3.3). The requirement that all neonates should pass all tests (HFT, ASR, TEOAE and DPOAE) may constitute a new reference standard for evaluating middle ear function in neonates. Previous studies [13, 14, 21, 22] had used a pass in DPOAE or TEOAE as the reference standard for normal middle ear function. Since passing OAE alone does not rule out minor middle ear dysfunction, a pass with a combination of tests was considered to provide a more stringent measure for normal middle ear function. It was reasoned that this stringent criteria may serve as a robust measure of middle ear function in neonates and provide the best reference standard available for newborns without resorting to invasive procedures such as myringotomy [32]. This stringent reference standard was established as it provides the best measure of middle ear function without resorting to invasive procedures.

There was no significant difference in absorbance between right and left ears at any of the frequencies in the present study. This finding agrees with that reported

by Hunter et al. [14] and Shahnaz [25]. On the contrary, Keefe et al. [24] found that at frequencies below 1.4 kHz, the left ear response had smaller absorbance than that of the right ear, whereas at frequencies above 1.4 kHz, the left ear response had larger absorbance than the right ear response. Keefe et al [24] attributed this difference between the ears to a relative (acoustic) stiffening of the left ear, with a resonance frequency in the neighbourhood of 1-1.4 kHz. Similarly, Merchant et al. [21] found small, albeit significant, differences between the mean measurements of the left and right ears. They reported that the right ear had larger absorbance than the left ear for the 0.5-1 kHz frequency range, while the left ear had larger absorbance than the right ear for the 1-4 kHz frequency range. Therefore, ear difference for absorbance measurements in neonates appears to be equivocal.

The present study did not find any gender difference for absorbance at any of the frequencies studied. This concurs with the findings of Hunter et al. [14] and Merchant et al. [21] who did not find a significant difference in absorbance between males and females. Conversely, Keefe et al. [24] found that at frequencies below 2 kHz, the absorbance in the male ear was smaller than that in the female ear. There was no gender difference at frequencies above 2 kHz. Keefe et al. [24] suggested that male ear is less stiff than female ears. The lack of agreement on gender differences between studies could be partially accounted for by the study sample. While the present study and studies by Hunter et al.[14] and Merchant et al.[21] included healthy neonates with normal middle ear function as measured against a reference standard, Keefe et al. [24] included neonates with risk factors (such as cleft lip and palate, and craniofacial anomalies) and used no measures to evaluate middle ear function.

Several studies have shown that, at all ages, absorbance is the lowest at frequencies below 1kHz Hz and above 4 kHz and highest in the frequency region between 1 and 4 kHz [13, 16-18, 21]. The present study is in agreement with these findings as the absorbance was high at 1.25-2 kHz and small at 0.3-0.8 kHz and 3-4 kHz. There was no significant difference in absorbance between the 1.25-2 kHz and 6-8 kHz regions. However, both inter-quartile and normative ranges were higher for frequencies above 2 kHz compared with frequencies below 2 kHz. The 6-8 kHz region had the highest inter-quartile and normative ranges. Due to this large variation,

the normative absorbance findings at these high frequencies may not provide useful clinical information to separate ears with and without middle ear disorders.

Although there was a considerable variation in absorbance with frequency, the diagnostic significance of each of the frequencies in the evaluation of middle ear pathology is yet to be determined. This process would involve evaluation of individual frequencies between 0.25 and 8 kHz. An alternate method would be to evaluate the AAI's obtained by grouping adjacent frequencies with similar absorbance. This method of AAI estimation, which involves fewer measurement variables, facilitates prompt and accurate interpretation of absorbance findings. Hunter et al. [14] and the present study have proposed two different sets of AAI's during the development of normative data. However, further research is needed to compare these AAI's with those obtained from neonates with middle ear pathology in order to evaluate their diagnostic significance.

The findings of the present study compare favourably with those of previous studies (Fig 3.2) [14, 25]. However, the present study showed slightly larger absorbance, especially from 1 to 2.5 kHz, when compared with these studies. The median values for Merchant et al. [21] and Sanford et al. [13] studies were not available for direct comparison with the present study. However, Merchant et al. [21] compared normative absorbance data for their neonatal subjects with other normative studies [13, 21, 24, 25]. The findings of the present study were also similar to those reported by Merchant et al. [21] but higher than those reported by Sanford et al. [13]. The differences in absorbance could be attributed to methodological differences among these studies. First, the reference standard used to determine the middle ear status was different. The present study used a test battery consisting of HFT, ASR, TEOAEs and DPOAEs to assess middle ear function. In contrast, other researchers have used either DPOAEs [13, 14, 21] or TEOAEs [25] as a reference standard to determine normal middle ear function. The use of a test battery approach would have ruled out minor middle ear dysfunction and considered only ears with normal middle ear function. The exclusion of subtle middle ear pathology possibly facilitates increased absorbance, as evident in the present study.

Second, the age of the study population differed across the studies. An age effect on WBA findings has been reported for infants in the first three months of life [17, 23]. Inclusion of NICU infants in their study sample increased the age at the time of testing in the studies by Shahnaz [25] and Keefe et al [24]. The present study and studies by Hunter et al. [14] and Sanford et al. [13] studied healthy neonates in the first few days of life. However, the average age of neonates at the time of testing in the present study was 46 h compared to 29 h in the study by Hunter et al [14] and 25.5 h in the study by Sanford et al. [13]. This could have contributed to the higher median absorbance for frequencies from 1 to 2.5 kHz in the present study compared to these studies [13, 14]. This observation also agrees with the findings of other studies [13, 21] that have noted increased absorbance on day 2 than day 1 due to the clearing of external and middle ear factors with age.

Third, the instruments used were different across the studies. The present study, Sanford et al. [13] and Keefe et al. [24] used Reftwin developed by Interacoustics A/S in Denmark, while Hunter et al. [14], Merchant et al. [21] and Shahnaz [25] used the Mimosa reflectance system. Different ear tips and calibration methods used between the two systems could have contributed to the observed differences between the studies [21, 22, 38].

Last, the method of ensuring a tight probe fit differed across the studies. The absorbance response is sensitive to the quality of probe fit which, in turn, affects the energy being reflected or absorbed. For example, the present study and the study by Hunter et al. [14] used a visual display of results during data acquisition, and the pattern of results of both studies concur with each other as well as with other studies on neonates and young neonates [21, 22, 25]. In contrast, Keefe et al. [24] used negative equivalent volume to verify the seal only during the recording of results. Using this method, Keefe et al. [24] reported that 13% of neonates in their study had poor acoustic seal. The relatively high absorbance at and below 1 kHz in their study also suggests poor acoustic seal in some ears. Therefore, probe fit should be checked during data acquisition using either visual display of results or equivalent volume to determine adequate seal. However, as Keefe et al. [24] suggest, there is a need for further experiments to compare the criterion for probe fit with the results obtained by an independent reference standard to check whether or not a leak is present.

3.6.1 Clinical application

The present study has provided normative WBA data by employing a very stringent reference standard to determine middle ear function. This stringent reference standard has ensured normal middle ear function and an efficient conductive mechanism as evidenced by increased absorbance in comparison to studies that have used only OAEs to determine normal middle ear status. Such a stringent reference standard can be used to compare ears with and without middle ear dysfunction to establish the sensitivity, specificity and efficacy of absorbance measurements in ears of neonates.

The results from the present study could be useful during assessment of middle ear function in neonates. Figures 3.3 and 3.4 show two case studies where this normative data can be applied. Figure 3.3 shows the absorbance values from a 76-h-old female neonate who passed the test battery. These absorbance values are within the normative range, suggesting normal middle ear function. Figure 3.4 shows the WBA from a 42-h-old female neonate who failed the test battery. Here, the absorbance values are below the normative range, suggesting middle ear dysfunction.

The present study defined the normative range as the range between the 10th and 90th percentiles. Further studies using test performance of WBA in ears with and without middle ear dysfunction are needed to determine whether this normative range is best suited for neonates. Additionally, this study used a test battery approach to ensure normal middle ear function. Among the tests in the battery, HFT, TEOAE and DPOAE require measurement at a single level and are easy to perform. On the contrary, ASR measurement involves presentation at various levels and the loud signal may disturb a well settled baby. Therefore, further research using test performance measures is necessary to determine the minimum test battery that should be used for the assessment of middle ear function in neonates.

3.6.2 Limitations of the study

Although normative data were obtained from healthy neonates who passed a battery of tests, a number of factors could have adversely affected the test results. First, the pass criteria were very stringent and, hence, only about one third (66 /195)

of neonates passed the test battery in one or both ears. The remaining two thirds of neonates either did not pass or had missing data with one or more tests in one or both ears. The ASR test was the most difficult to perform and, most often, the neonates either failed the ASR test or the ASR test could not be completed as the neonate was unsettled. Second, the activity state of the neonates affected the measurement of WBA. In a well settled neonate, the test could be completed within 10 s. However, if the neonate was unsettled or being fed, the high physiologic noise generated by the neonate would invalidate the WBA results. This factor was controlled by measuring WBA when the neonate was well settled. Third, it was not always possible to get a tight probe seal in the neonates. In some circumstances, the probe had to be removed and reinserted more than once to obtain a good seal during the test. This process had the potential to disturb the well settled neonate. A visual display of the probe seal was checked with every neonate to ensure that there was no reduced reflectance in the frequencies below 1000 Hz due to leakage.

3.6.3 Summary

In conclusion, the present study provided normative wideband absorbance data in healthy neonates who passed HFT, ASR, TEOAE and DPOAE. The results of this study showed higher absorbance and more efficient middle ear transfer function compared to other studies that have obtained normative WBA using only OAEs as the reference standard. The normative data obtained in this study can be applied clinically to assess middle ear function in neonates. Further research using test performance measures is needed to determine the best combination of tests required to establish a reference standard.

3.7 Acknowledgements

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Chapter Four: Wideband Absorbance Outcomes In Newborns: A Comparison With High Frequency Tympanometry, Automated Brainstem Response, Transient Evoked And Distortion Product Otoacoustic Emissions.

4.1 Background

Determination of true status of the outer and middle ear is very difficult with neonates and young infants. Apart from air and bone conduction tone burst ABR (AC & BC TB ABR) threshold measures, there is no gold standard for evaluating the function of the conductive pathway in this population. However, AC and BC TB ABR measures are time consuming and not commonly used in newborn hearing screening programs. In previous studies of wideband absorbance (WBA), distortion product otoacoustic emissions (DPOAE) have been used as a reference standard. Since DPOAE cannot reliably identify the true outer and middle ear conditions, the conclusions that can be drawn regarding the diagnostic accuracy of WBA are limited. Use of DPOAE as a reference standard in the evaluation of diagnostic accuracy of WBA is a serious shortcoming and limits the clinical applicability of WBA.

This study compared the test performance of WBA against four single test and three composite test (or test battery) reference standards. The aim was to determine whether WBA can provide a more effective alternate to either individual tests or a combination of tests for determining the outer and middle ear status. The results of this study are presented in Chapter Four of this thesis.

Chapter Four of this thesis, entitled, “Wideband absorbance outcomes in newborns: A comparison with high frequency tympanometry, automated brainstem response, transient evoked and distortion product otoacoustic emissions” is based on an article submitted for publication in *Ear and Hearing*. While this manuscript is inserted as a chapter in this thesis, the formatting of section sub-headings and numbering of tables and figures have been modified from the original publication to

match the thesis format. The referencing of the article is retained as per the *Ear and Hearing* journal format.

Aithal, S., Kei, J., Driscoll C., & Khan, A. Wideband absorbance outcomes in newborns: A comparison with high frequency tympanometry, automated brainstem response, transient evoked and distortion product otoacoustic emissions. Article submitted to Ear and Hearing.

4.2 Abstract

Objectives: The purpose of this study was to evaluate the test performance of wideband absorbance (WBA) in terms of its ability to predict the outer and middle ear status as determined by nine reference standards.

Design: Automated auditory brainstem response (AABR), high frequency (1000 Hz) tympanometry (HFT), transient evoked otoacoustic emission (TEOAE) and distortion product otoacoustic emission (DPOAE) tests were performed on 298 ears (144 right, 154 left) of 192 (108 males, 84 females) neonates with a mean age of 43.7 hours (SD = 21.3, range = 8.3 to 152.2 hours). WBA was measured from 0.25 to 8 kHz using clicks under ambient pressure conditions. Test performance of WBA was assessed in terms of its ability to identify conductive conditions in neonates when compared with nine reference standards (including four single tests and five test batteries) using the receiver operating characteristic (ROC) analysis.

Results: The test performance of WBA against the test battery reference standards was better than that against single test reference standards (AABR, HFT, TEOAE and DPOAE). The area under the ROC curve reached a high value of 0.78 for HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE reference standards. Within the ears that passed each of the reference standards, there were no significant differences in WBA. However, for the ears that failed each of the test standards, there were significant differences in WBA. The region between 1 and 4 kHz provided the best discriminability to evaluate the conductive status compared to other frequencies.

Conclusions: WBA is a desirable measure of conductive conditions in newborns due to its superior test performance in classifying ears that passed or were referred, using the above composite standards' (HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE) test outcomes as the surrogate gold standard compared to single test standards.

4.3 Introduction

Assessment of outer and middle ear function is important in differentiating a transient conductive condition from a sensorineural condition. Conventional tympanometry, a standard test of middle ear function used with children and adults, has limited application with neonates. Tympanometry using a 226-Hz probe tone has been found to be insensitive to conductive conditions in newborns (Paradise et al. 1976; Marchant et al. 1984; Hunter & Margolis 1992; Kei et al. 2003). Although high frequency tympanometry (HFT) using a 1-kHz probe tone is recommended for use with young infants (Kei et al. 2003; Margolis et al. 2003; Baldwin 2006; Alaerts et al. 2007), there are no universally agreed methods for interpreting results (Kei & Mazlan 2012).

Auditory brainstem response (ABR) is currently considered as the gold standard measure for assessing the auditory function of infants referred from newborn hearing screening. A combination of air conduction (AC) and bone conduction (BC) ABR for tone burst stimuli has been shown to be useful in determining the type and degree of hearing loss (Prieve et al. 2013). However, Stapells (2011) reported that many clinicians do not routinely obtain ABR results for bone conduction stimuli after finding elevated AC thresholds. Instead, they rely on immittance results for determining the middle ear status. Stapells also reported that when BC testing is performed, the results are not used to calculate the air-bone gap but are primarily used to indicate whether BC thresholds are normal or elevated.

An alternate method of outer and middle ear assessment is wideband acoustic immittance (WAI) which includes measures such as wideband reflectance (WBR) and wideband absorbance (WBA). WBR is defined as the ratio of reflected power to incident power (Voss & Allen 1994). WBA, defined as $1 - \text{WBR}$, represents the proportion of sound energy absorbed by the middle ear using a wideband stimulus (such as a click or chirp) which covers a frequency range from 0.2 to 8 kHz. WBA varies from 1.0 meaning that all the energy is absorbed by the middle ear to 0.0 meaning that all energy is reflected from the middle ear (Feeney & Sanford 2012). In the present paper, the findings of all studies will be discussed in terms of WBA for ease of comparison with the present study.

Studies have shown WBA to be sensitive to various conductive disorders in children (Jeng et al. 1999; Piskorski et al. 1999; Keefe & Simmons 2003; Beers et al. 2010; Keefe et al. 2012) and adults (Feeney et al. 2003; Keefe & Simmons 2003; Allen et al. 2005). WBA is also reported to be useful in the neonatal population (Keefe et al. 2003; Vander Werff et al. 2007; Sanford et al. 2009; Hunter et al. 2010). More importantly, Keefe et al. (2003) demonstrated that inclusion of WBA test in universal newborn hearing screening (UNHS) programs decreased the false positive rates from 5% to 1%, thus, suggesting that information on outer and middle ear status improves the ability to predict hearing status. Consequently, WBA is recommended as an adjunct tool in UNHS programs (Vander Werff et al. 2007; Sanford et al. 2009; Hunter et al. 2010; Merchant et al. 2010; Werner et al. 2010; Feeney & Sanford 2012).

In addition, WBA has been found to predict distortion product otoacoustic emission (DPOAE) outcomes more accurately than HFT (Sanford et al. 2009; Hunter et al. 2010). Sanford et al. (2009) investigated test performance of WBA and HFT in terms of their ability to predict DPOAE outcomes in 455 neonate ears. They reported an area under the receiving operating characteristic curve (AROC) of 0.87 for WBA and 0.75 for HFT. Similarly, Hunter et al. (2010) studied the test performance of WBA and HFT to predict DPOAE outcomes in 324 neonates and reported an AROC of 0.90 for WBA at 2 kHz, 0.82 for WBA at 1 kHz and 0.72 for HFT.

Although WBA has been reported to be useful in predicting outer and middle ear status in neonates, evaluating its test performance is challenging. Even though air and bone conduction (AC and BC) tone burst ABR may be regarded as a surrogate gold standard for detecting conductive conditions, this threshold ABR measure requires long testing time to complete and is done as a diagnostic measure at some point later in time rather than during the hearing screening period. Furthermore, as Sangster (2011) suggests, most studies circumvent this issue by using DPOAE as the reference standard because it is already used in screening programs and it indirectly gives information on the forward and reverse transmission of sound through the middle ear. However, DPOAE may not accurately identify minor or sub-clinical conductive pathologies (Kemp 2002) and DPOAE outcomes are affected by

physiologic and ambient noise. Hence, it may not serve as an ideal reference standard (Sanford et al., 2009; Hunter et al., 2010). This represents a significant shortcoming in the evaluation of WBA, and limits its clinical applicability (Sangster 2011).

A strict gold standard to determine middle ear effusion would be myringotomy. Since ethical considerations prohibit surgical procedures in non-symptomatic neonates, myringotomy cannot be used to determine the test performance of WBA. In light of this limitation, an alternate, albeit less strict, reference standard based on the outcomes of a battery of tests, may be used instead (Mazlan & Kei 2012). Aithal et al. (2013) developed normative WBA data on neonates using a test battery consisting of automated auditory brainstem response (AABR), HFT, acoustic stapedial reflex (ASR), transient evoked otoacoustic emission (TEOAE) and DPOAE tests as a reference standard to determine the outer and middle ear status. They suggested that this composite reference standard provided the best measure of outer and middle ear function without resorting to invasive procedures. Nevertheless, an important clinical question is how WBA compares with various individual tests (such as TEOAE and HFT) or composite of tests in terms of predicting test outcomes in the neonatal population.

The aim of the study was to evaluate the test performance of WBA against nine reference standards consisting of four single tests and five test batteries in an attempt to determine whether WBA can provide a more effective alternate to either individual tests or a combination of tests for determining the outer and middle ear status in neonates.

4.4 Method

The present study enrolled 192 healthy neonates born at the maternity unit of The Townsville Hospital. The Townsville Health Service District Institutional Ethics Committee and the University of Queensland Behavioural and Social Sciences Ethical Review Committees approved the study. Written consent was obtained from the parents during their hospital stay.

4.4.1 Subjects and test environment

In total, 192 (108 males, 84 females) healthy neonates were recruited for the present study. Of these infants, 154 were Caucasian, 31 were Aboriginal, 5 were Asian and 2 were African. None of the infants included in the present study participated in the Aithal et al. (2013) study. Mean age of the neonates at the time of testing was 43.7 hours (SD= 21.3, range = 8.3 to 152.2 hours). Only three neonates were more than 100 hours old. Mean gestational age of the neonates was 39.2 weeks (SD = 1.2, range = 36-42 weeks). Mean birth weight was 3476.9 g (SD= 460.9, range = 2290-5000 g).

All measurements were performed in a quiet room in the maternity unit. Nursing staff performed AABR screen while an experienced audiologist performed the other tests. AABR screen was always performed first followed by HFT, TEOAE, DPOAE and WBA tests, in no particular order. The order of testing depended on the activity state of the neonate and how feasible it was to obtain a good seal for performing the test. The most accessible ear was tested first and the second ear was tested if the neonate was well settled and there was adequate time for testing.

4.4.2 Procedure

4.4.2.1 Automated auditory brainstem response: The AABR screening was done using the Natus ALGO3. Click stimuli were presented at a level of 35 dB nHL to both ears via ear muffins. A pass or refer for each ear was automatically displayed on the screen.

4.4.2.2 High frequency tympanometry: HFT was performed using a Madsen Otoflex 100 acoustic immittance device with a 1-kHz probe tone. The pressure was changed from +200 to -400 daPa at a rate of 400 daPa/sec and admittance (Y) tympanograms were obtained. The pass criterion was a single positively peaked tympanogram with peak compensated static admittance (+200 daPa tail to peak) of more than 0.2 mmho (Mazlan et al. 2009).

4.4.2.3 Transient evoked otoacoustic emissions: TEOAEs were obtained using a Biologic Navigator Plus. The signal consisted of wideband clicks of 80 μ s duration, at a targeted amplitude of 80 dB pkSPL. The pass criteria included

reproducibility of at least 70% and a signal-to-noise ratio (SNR) of at least 3 dB at 2, 3, and 4 kHz (Kei et al. 2003; Vander Werff et al. 2007).

4.4.2.4 Distortion product otoacoustic emissions: DPOAEs were obtained using the same Biologic device as the TEOAEs. DPOAEs were measured in response to stimulation using pairs of primary tones (F1, F2) with F2 frequency set at 2, 3, 4, and 6 kHz with a frequency ratio (F2/F1) of 1.2 and fixed levels of 65 and 55 dB SPL for F1 and F2, respectively. Pass criteria included (i) the noise level less than 0 dB SPL and (ii) the SNR to be a minimum of 6 dB in at least three out of four F2 frequencies (Sanford et al. 2009; Hunter et al. 2010) and (iii) a DPOAE magnitude with a level of at least -6 dB SPL (Sanford et al. 2009; Merchant et al. 2010).

4.4.2.5 Wideband absorbance: WBA was measured using a prototype research system developed by Interacoustics A/S (Denmark). The ReFlwin computerised system consisted of a Windows-based computer, a 24-bit resolution sound card, a pressure pump and controller system contained in an acoustic immittance instrument (AT235), and custom software (version 3.2.1) for stimulus generation and data acquisition. Calibration was performed every day prior to data collection. Keefe and Simmons (2003) provide a detailed description of the calibration process for WBA. Calibration of the ReFlwin system was based on the analysis of wave characteristics within two rigid walled cylindrical calibration tubes 232.3 and 5.6 cm in length. A calibration was accepted as long as the root-mean-squared reflectance error (ΔR) did not exceed 0.009 and the loss parameter χ was in the range from 1 to 1.09 (Keefe & Simmons 2003; Sanford et al. 2009).

WBA measurements at ambient pressure were obtained by recording acoustic responses to clicks, presented at 55 dB SPL and at a rate of one click per 46 msec to the neonate's ear. Responses from a total of 16 clicks were averaged for each measurement and WBA was calculated for each averaged response. A visual display with high absorbance at frequencies below 1 kHz served as an on-screen prompt that alerted the tester if there was a probe leak. Absorbance of 0.7 or more at each of the six one-third octave frequencies between 0.25 and 0.8 kHz (0.25, 0.3, 0.4, 0.5, 0.6 and 0.8 kHz) was considered high and was suggestive of a probe leak. When this

happened, the probe was removed and re-inserted to ensure that an adequate seal was obtained for another WBA measure. The typical test time for the WBA test was less than 10 seconds for a quiet neonate.

4.4.3 Reference standards and pass/fail classification

Presently, there is no unanimous agreement on which reference standard should be used to determine the test performance of WBA. For instance, Sanford et al. (2009) and Hunter et al. (2010) have used DPOAE while Vander Werff et al. (2007), Shahnaz (2008) and Silva et al. (2013) have used TEOAE to determine the status of the middle ear. Although Norton et al. (2000) have found the performance of TEOAE and DPOAE to be similar in a UNHS program, the inclusion of both TEOAE and DPOAE as reference standards would be useful because the mechanisms involved in generating otoacoustic emissions by the two procedures are different and the two procedures demonstrate different susceptibility to noise, resulting in different test outcomes in the individuals (Costa et al 2009; Rhoades et al. 1998; Shi et al. 2000). In the present study, nine reference standards were established using the AABR, HFT, TEOAE and DPOAE tests or their combinations for the determination of test performance of WBA. These reference standards are presented in Table 1. The reason for including a combination of tests as reference standards was the assumption that neonates who passed a battery of tests involving HFT, TEOAE and DPOAE were more likely to have a normal conductive pathway (outer and middle ear) than those who passed a single test (Aithal et al. 2013).

Table 4.1 Single test/test battery reference standards adopted in the present study

Reference Standard
Automated auditory brainstem response (AABR)
High frequency tympanometry (HFT)
Transient evoked otoacoustic emissions (TEOAE)
Distortion product otoacoustic emissions (DPOAE)
HFT + TEOAE
HFT + DPOAE
TEOAE+DPOAE
HFT + TEOAE + DPOAE
AABR+TEOAE+DPOAE

The pass criteria for single tests were determined as described earlier in this section. A strict parallel test protocol (Turner et al. 1999) was used with the test battery reference standards. With this criteria, the ear with a pass in all the tests in a particular test battery was included in the ‘pass’ condition for that reference standard. For instance, with the HFT+TEOAE reference standard, only ears with a pass in both HFT and TEOAE tests were included in the pass condition for that test battery while the results of the other individual tests were not considered. Likewise, the test protocol for neonates who failed in a test battery reference standard was also a strict parallel protocol wherein an ear with a fail in those tests in the test battery was included in the ‘fail’ condition for that reference standard. For example, with the HFT+TEOAE+DPOAE reference standard, only the ears with a fail in all three individual tests (HFT, TEOAE and DPOAE) were included in the fail condition for that reference standard.

Table 2 shows different reference standard tests/test batteries with the number of ears that passed or failed against each reference standard. Only the ears that either passed or failed individual tests, or all tests in the case of test batteries, were included for analysis. With the strict test protocol, the ears that passed some but not all the tests were not included for analysis. Only one test battery that included AABR was considered for analysis. This is because, only 10 out of 298 ears included in the study failed AABR. When AABR was included along with other tests in the test batteries, the number of ears that failed the respective test battery was further reduced. For instance, only four ears failed (243 passed) AABR+HFT and AABR+DPOAE, nine ears failed (268 passed) AABR+TEOAE and only two ears failed (200 passed) AABR+HFT+TEOAE+DPOAE reference tests. Nevertheless, only results for AABR+TEOAE+DPOAE are provided for comparison against other reference test standards.

The pass/fail classification for the WBA measure was more complicated than that of other tests because of the large number of frequencies measured. In the present study, WBA was measured at 16 single frequencies from 0.25 to 8 kHz in one-third octave intervals. Using the same equipment but different subjects, Aithal et al. (2013) developed normative WBA data at the 16 frequencies for newborns using a pass in a

test battery consisting of AABR, HFT, ASR, TEOAE and DPOAE as a reference standard. They suggested that this reference standard provided the best measure of outer and middle ear function without resorting to an invasive surgical procedure such as myringotomy. The normative range of WBA at each frequency, as described by Aithal et al. (2013), was used to determine pass/fail criteria for the neonates. WBA values at and above the 10th percentile of the normative data were considered as a pass, whereas values below the 10th percentile were considered as a fail. WBA data was available for all the 298 ears included in the study. Table 3 shows the number of ears that passed or failed WBA.

Table 4.2 Number of ears that passed or failed in nine reference standards

Reference standard	Number of ears that passed	Number of ears that failed	Total number of ears
AABR	288	10	298
HFT	186	89	275
TEOAE	206	88	294
DPOAE	218	57	275
HFT + TEOAE	147	45	192
HFT + DPOAE	150	27	177
TEOAE+DPOAE	235	52	183
HFT + TEOAE + DPOAE	134	21	155
AABR+TEOAE+DPOAE	183	8	191

Table 4.3 Number of ears that passed or failed WBA at various frequencies

Frequency (kHz)	Pass	Fail
0.25	242	56
0.3	266	32
0.4	261	37
0.5	258	40
0.6	268	30
0.8	259	39
1	257	41
1.25	253	45
1.5	253	45
2	256	42
2.5	257	41
3	269	29
4	253	45
5	244	54
6	258	40
8	257	41

4.4.4 Statistical Analysis

To compare WBA among the nine reference standards, an analysis of variance (ANOVA) was performed with WBA as the dependent variable, and frequency (16 levels) and reference standard as independent variables. An alpha level of 0.05 was used for all analyses. *Post hoc* analysis using Bonferroni correction was performed to examine the effect of reference standard on WBA. ANOVA was also performed to determine the significance of difference between pass and fail conditions for each reference standard.

Receiver operating characteristic (ROC) curves, showing test sensitivity against one minus test specificity, are a standard procedure to evaluate the test performance of a diagnostic test. They show to what extent two distributions (e.g., pass and fail) overlap. The further apart the distributions, the greater will be the area

under the ROC curve (AROC), which is an overall indication of the diagnostic accuracy of a ROC curve (Zhou et al. 2002). An AROC value of 1 indicates that the test measure reliably distinguishes between two mutually exclusive distributions, for instance, 'normal' and 'disease' conditions. On the other hand, an AROC value of 0.5 indicates that the predictor is no better than chance to determine the conditions (Zhou et al. 2002). Test performance for all the nine reference standards was determined using SPSS software (version 20). Additionally, AROC was determined for various absorbance area indices (0.25-0.3, 0.4-0.8, 1, 1.25-2, 2.5, 3-4 and 4-8 kHz) as described by Aithal et al. (2013). Statistical significance of difference between the AROCs was determined as suggested by Hanley and McNeil (1982).

4.5 Results

Figure 4.1 displays the results for median WBA for the 'pass' condition for the nine reference standards along with the normative range (10th to 90th percentile) provided by Aithal et al (2013). The median WBA from 0.25 to 8 kHz was similar across all reference standard pass conditions with two maxima occurring at 1.5 and 6 kHz. The WBA ranged between 0.40 and 0.76 across all reference standard pass conditions. The WBA for all reference standards was within the normative range provided by Aithal et al. (2013).

An ANOVA with repeated measures was applied to the data with WBA as the dependent variable, and frequency and reference standard as independent variables. The results revealed a significant frequency effect [$F(8, 13925) = 7.31, p = 0.00$]. However, the difference in WBA across reference standards did not reach significance [$F(66, 13925) = 0.97, p = 0.55$]. There was no significant interaction between frequency and reference standard [$F(8, 1686) = 1.89, p = 0.06$]. Further analysis of the frequency effect was not conducted since WBA pattern across the frequency range was similar to that reported by earlier normative studies, wherein absorbance was highest in the frequency region between 1 and 4 kHz and lowest at frequencies below 1 kHz and above 4 kHz (Keefe et al. 1993; Sanford et al. 2009; Hunter et al. 2010; Merchant et al. 2010; Aithal et al. 2013).

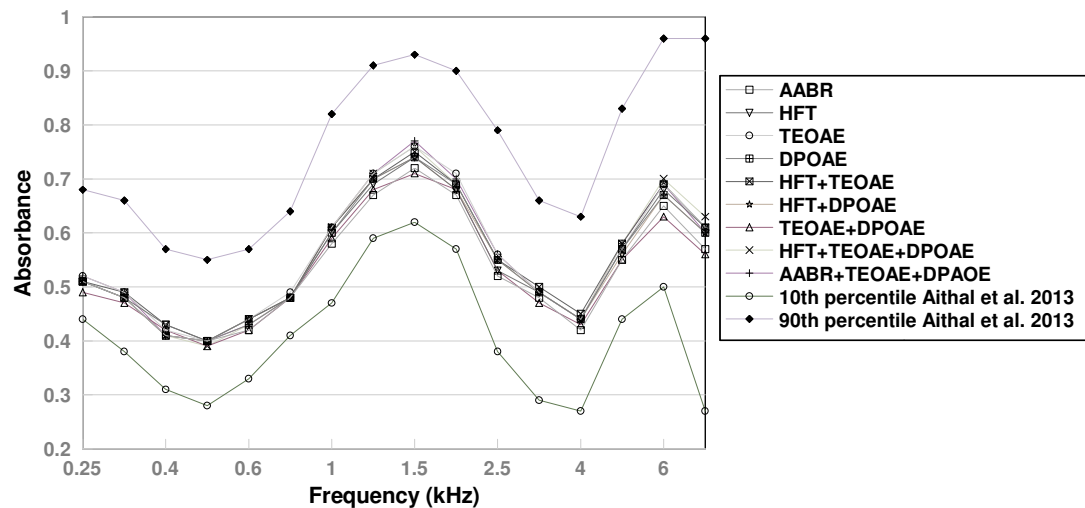


Figure 4.1: Median absorbance obtained from ears that passed various reference standards plotted against frequency in comparison to the normative range obtained by Aithal et al (2013).

Figure 4.2 illustrates the standard deviation (SD) of WBA across nine reference standards for the pass condition. Across all reference standards, SD was the least (0.12-0.17) between 0.25 and 0.8 kHz, medium (0.16-0.23) between 1 and 3 kHz and highest (0.26-0.28) at 8 kHz. There was a general trend for SD to increase with frequency from 0.8 to 8 kHz.

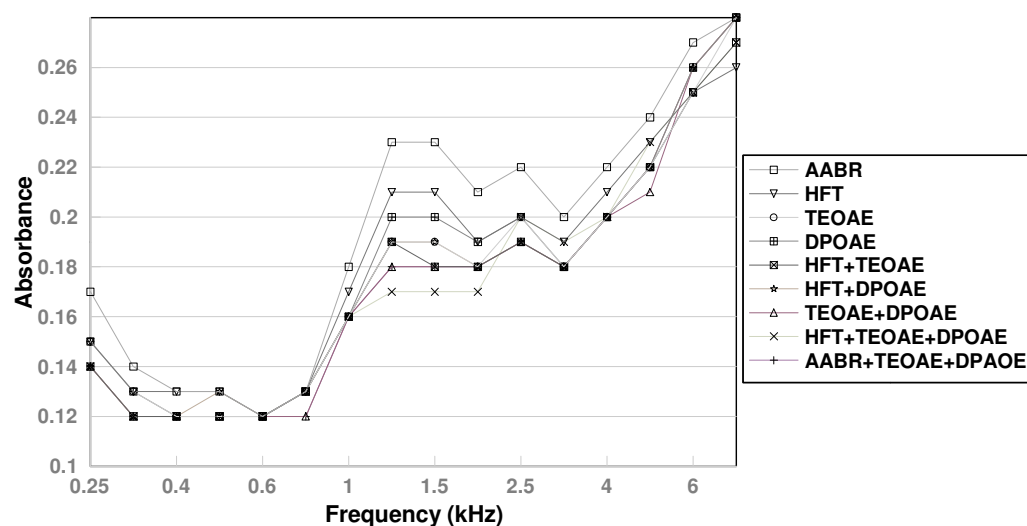


Figure 4.2: Standard deviation of absorbance for ears that passed various reference standards

Median WBA data across nine reference standards for the pass condition in the present study were compared with normative data provided by Shahnaz (2008), Sanford et al. (2009), Hunter et al. (2010), Merchant et al. (2010) and Aithal et al. (2013). As illustrated in Figure 4.3, apart from the difference in magnitude of WBA, the pattern of WBA was similar across the present study and other studies in that WBA was high between 1 and 4 kHz and reduced below 1 kHz and above 4 kHz.

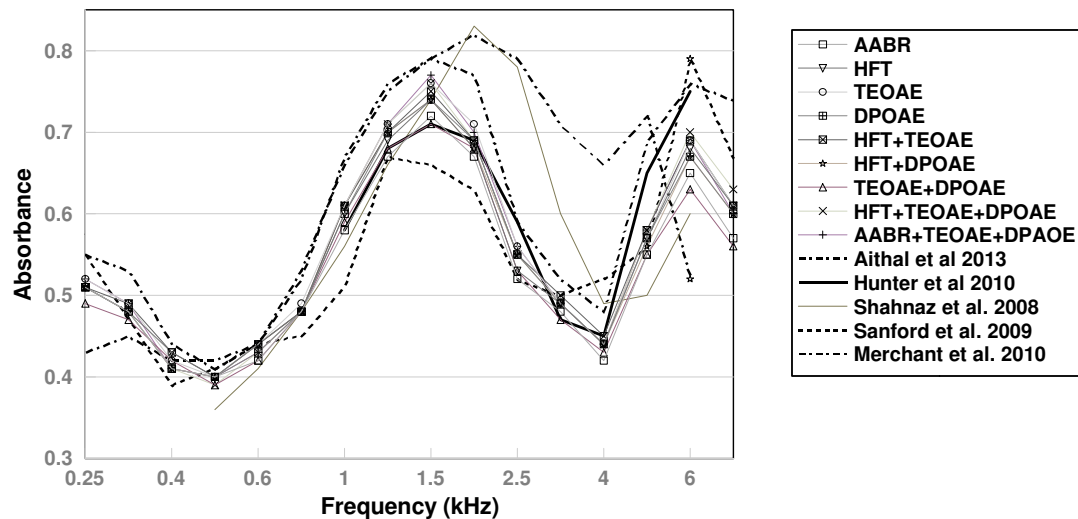


Figure 4.3: Median absorbance obtained from ears that passed various reference standards plotted against frequency in comparison to five published studies

Median WBA values for the ‘fail’ condition for the nine reference standards are displayed in Figure 4.4. The normative region (10th and 90th percentiles) described by Aithal et al. (2013) is also plotted in this figure for comparison. Apart from the median WBA pattern for the HFT reference standard which showed a large peak at 1.5 kHz, the traces corresponding to the other reference standards were relatively flat across the entire frequency range with minor peaks and troughs from 1 to 8 kHz. The median WBA values for all, except the HFT, reference standards were below the 10th percentile values obtained by Aithal et al. (2013).

Figure 4.5 illustrates the standard deviation (SD) of WBA across nine reference standards for the fail condition. While there was a general trend for SD to increase with increasing frequency, the SD values for the fail condition were generally greater than those for the pass condition (Figure 4.2).

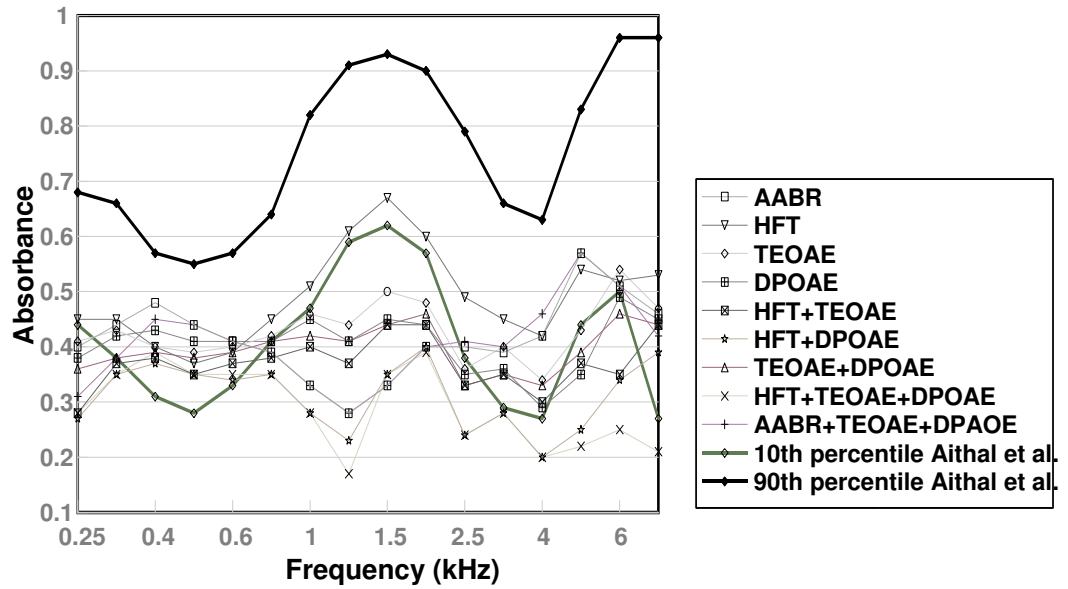


Figure 4.4: Median absorbance obtained from ears that failed various reference standards plotted against frequency in comparison to the normative range obtained by Aithal et al (2013).

Table 4.4 Results of a *post hoc* analysis using multiple comparisons with Bonferroni correction comparing wideband absorbance averaged across all frequencies between reference standards for ears that failed each of the reference standards.

	HFT	TEOAE	DPOAE	HFT+ TEOAE	HFT+ DPOAE	TEOAE+ DPOAE	HFT+ TEOAE+ DPOAE	AABR+ TEOAE+ DPOAE
AABR	0.36	0.75	1.00	0.64	0.35	0.98	0.29	0.92
HFT		0.22	0.06	0.01*	0.00*	0.00*	0.08	0.34
TEOAE			0.53	0.14	0.03*	0.02*	0.50	0.67
DPOAE				0.41	0.25	0.09	0.96	0.90
HFT+ TEOAE					0.45	0.33	0.44	0.76
HFT+ DPOAE						0.79	0.14	0.45
TEOAE+ DPOAE							0.10	0.39
HFT+ TEOAE+ DPOAE								0.92

* - denotes significant difference with $p < 0.05$

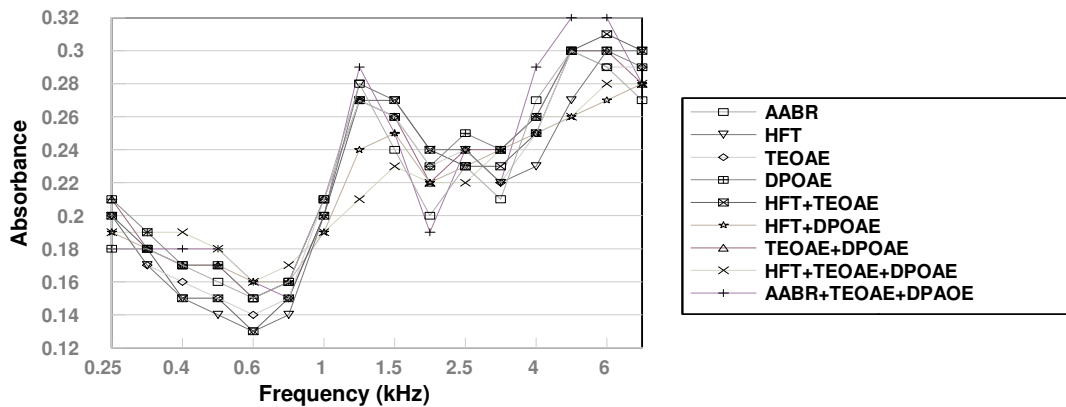


Figure 4.5: Standard deviation of absorbance for ears that failed various reference standards

The WBA results obtained from ears that failed in the reference standards were analysed using an ANOVA with repeated measures with WBA as the dependent variable, and frequency and reference standard as independent variables. The results revealed a significant effect for frequency [$F(7, 2756) = 17.23, p = 0.00$] and reference standard [$F(8, 388) = 3.82, p = 0.03$]. The interaction between frequency and reference standard [$F(56, 2756) = 0.88, p = 0.72$] was not significant. A *post hoc* analysis using multiple comparisons with Bonferroni correction was performed to analyse the effect of reference standards. As seen in Table 4.4, the results revealed significant difference in WBA averaged across all frequencies between HFT and three test battery reference standards (HFT+TEOAE, HFT+DPOAE and TEOAE+DPOAE), and between TEOAE and two test battery reference standards (HFT+DPOAE and TEOAE+DPOAE). However, there were no significant differences in WBA between the other reference standards.

To ascertain if WBA obtained from ears that failed in each reference standard vary with frequency, a *post hoc* analysis using multiple comparisons with Bonferroni correction was applied to the data. The results showed no significant difference in WBA across frequencies for AABR, HFT, HFT+DPOAE, HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE tests, indicating a relatively flat response pattern for these reference standards. WBA was significantly different between 0.6 kHz and

0.25, 1.5, and 5 kHz for the TEOAE+DPOAE test battery, and between 0.25 and 0.6 kHz only for the HFT+TEOAE test battery. For the TEOAE test, WBA was significantly different between 0.25 and 0.6 kHz, between 0.5 and 1.25 kHz, and between 3 kHz and 1.5, 2.5, 4 and 5 kHz. For the DPOAE test, WBA was significantly different between 1.25 and 0.3, 0.4 and 0.5 kHz, between 2 kHz and 0.3, 0.4, 0.5, 0.6, and between 3 and 8 kHz, indicating significant variations in the WBA response.

As seen from Figures 4.1 and 4.4, the median WBA for the fail condition was generally lower than that for the pass condition for each reference standard. Table 4.5 demonstrates the significance of the difference in WBA between the pass and fail condition for each reference standard. For all reference standards, WBA condition was significantly different from 0.8 to 2.5 kHz across the two conditions. A close examination of results revealed that WBA for pass condition was significantly different from the WBA for fail condition across all frequencies between 0.25 and 8 kHz for the test battery reference standards except for TEOAE+DPOAE and AABR+TEOAE+DPOAE.

An AROC was computed to determine the test performance of WBA against each reference standard. The results for the nine reference standards are shown in Table 4.6. In general, across all the reference standards, AROC was the highest at 1.25 kHz. For instance, the AROC at 1.25 kHz was the highest for, HFT+TEOAE+DPOAE (0.78) AABR+TEOAE+DPOAE (0.78) and HFT+DPOAE (0.77). In comparison, AROC for other reference standards ranged between 0.57 and 0.73 at 1.25 kHz. Hunter et al. (2010) found that 2 kHz was the best absorbance frequency for discriminating DPOAE pass from DPOAE refer conditions. The results from Table 4.6 show that the AROC of WBA at 2 kHz against TEOAE at 2 kHz was 0.63 and against DPOAE at 2 kHz was 0.63.

Table 4.5: Significance of difference in WBA between the pass and fail conditions for nine reference standards.

Frequency (kHz)					HFT+	HFT+	TEOAE+	HFT+	AABR+
	AABR	HFT	TEOAE	DPOAE	TEOAE	DPOAE	DPOAE	TEOAE+	TEOAE+
0.25	0.03*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
0.3	0.25	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.02*
0.4	0.93	0.04*	0.07	0.42	0.00*	0.00*	0.17	0.00*	0.53
0.5	0.96	0.04*	0.24	0.99	0.00*	0.02*	0.58	0.01*	0.71
0.6	0.40	0.02*	0.02*	0.25	0.00*	0.00*	0.06	0.03*	0.21
0.8	0.07*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.01*
1	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
1.25	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
1.5	0.00*	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
2	0.00*	0.03*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
2.5	0.00*	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
3	0.16	0.06	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.20
4	0.77	0.06	0.01*	0.02*	0.00*	0.00*	0.00*	0.00*	0.90
5	0.39	0.02*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.34
6	0.29	0.01*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.12
8	0.32	0.04*	0.01*	0.01*	0.00*	0.00*	0.00*	0.00*	0.04*

* - denotes significant difference with $p < 0.05$

Figures 4.6 and 4.7 illustrate examples of ROC curves for WBA across 16 one-third octave frequencies for an individual test (DPOAE) and test battery (HFT+TEOAE+DPOAE) reference standards, respectively. These figures demonstrate higher AROC for WBA against test battery reference standard compared to DPOAE reference standard across all 16 frequencies.

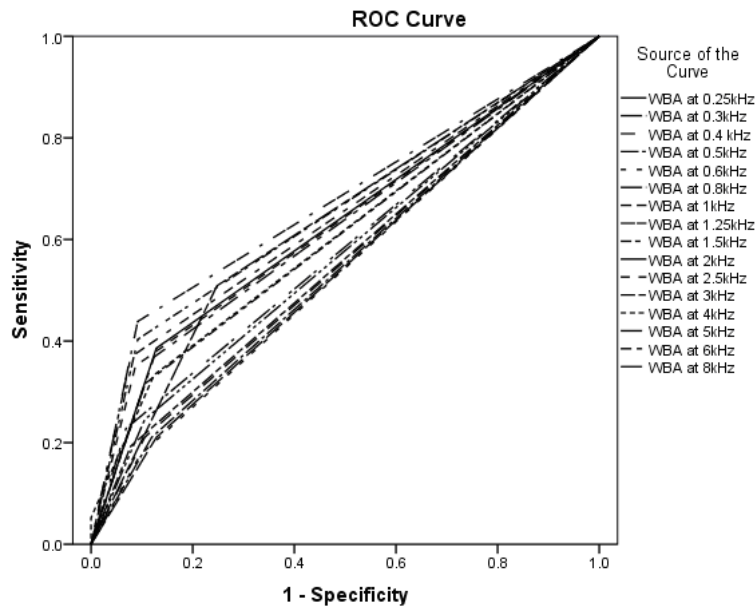


Figure 4.6: ROC curves for WBA against DPOAE reference standard

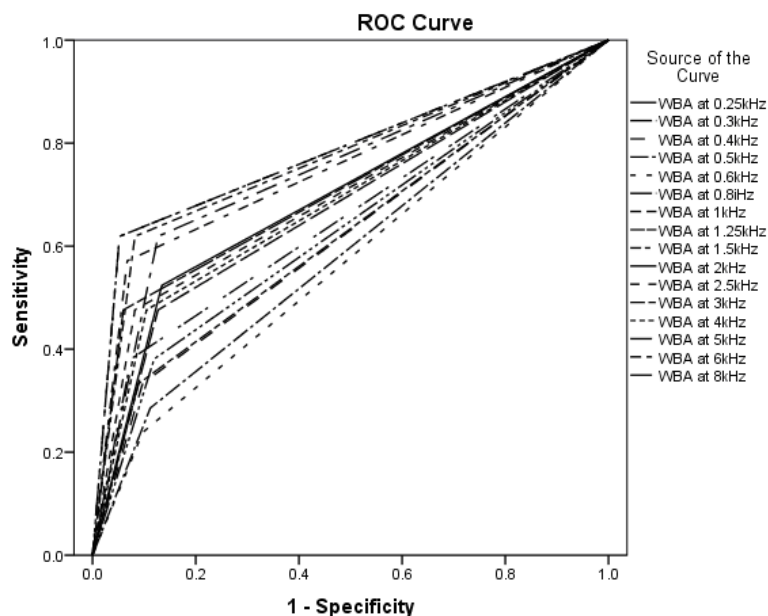


Figure 4.7: ROC curves for WBA against HFT+TEOAE+DPOAE reference standard

To determine whether the AROC was significantly different from 0.5 at each of the 16 frequencies for all reference standards, a statistical test as described by Hanley and McNeil (1982) was applied. As illustrated in Table 4.6, the AROC was significantly different from 0.5 in the frequency range between 1 and 6 kHz and between 0.25 and 0.3 kHz for all the reference standards except HFT, AABR and AABR+TEOAE+DPOAE tests.

Furthermore, WBA was also determined for various absorbance area indices (AAIs) as described by Aithal et al (2013). Table 4.7 illustrates the AROC for seven AAIs for each reference standard. As seen in Table 4.7, the AROC for WBA was significantly different from 0.5 across all AAIs for the HFT+TEOAE test battery. The AROC for WBA was significantly different from 0.5 for the 1.25-2 kHz AAI for all reference standards except for the HFT. Interestingly, the AROCs for WBA at 1.25-2 kHz for these reference standards attained maximum values when compared to other AAIs.

In addition, significance of difference between AROCs for WBA against different reference standards was determined for each AAI using Hanley and McNeil's (1982) method. There were no significant differences in AROCs between any two reference standards for the AAI of 0.25-0.3, 0.4-0.8, 2.5, and 3-4 kHz ($p > 0.05$). At 1 kHz AAI, there was a significant difference in AROC for WBA between HFT and HFT+DPOAE ($p = 0.01$), and between HFT and HFT+TEOAE+DPOAE ($p = 0.02$) reference standards. At 1.25-2 kHz AAI, AROC for WBA against HFT was significantly lower than that for TEOAE ($p = 0.01$), DPOAE ($p = 0.01$), HFT+TEOAE ($p = 0.01$), HFT+DPOAE ($p = 0.00$), TEOAE+DPOAE ($p = 0.00$) and HFT+TEOAE+DPOAE ($p = 0.00$). At 5-8 kHz AAI, the AROC for WBA against HFT was significantly different from WBA against that for HFT+DPOAE ($p = 0.04$) and HFT+TEOAE+DPOAE ($p = 0.01$) reference standards. AROC for WBA against TEOAE was also significantly different from that for HFT+DPOAE ($p = 0.02$) and HFT+TEOAE+DPAOE ($p = 0.01$) reference standards. There was no significant difference between WBA for AABR and other eight reference standards.

Table 4.6 Test performance of wideband absorbance as judged by area under the receiver operating characteristic curves against nine reference standards

Frequency (Hz)	AABR	HFT	TEOAE	DPOAE	HFT + TEOAE	HFT + DPOAE	TEOAE+ DPOAE	HFT + TEOAE+ DPOAE	AABR +TEOAE +DPOAE
0.25k	0.61	0.58*	0.62*	0.64*	0.69*	0.74*	0.64*	0.70*	0.69
0.3k	0.54	0.55	0.58*	0.58	0.63*	0.65*	0.59*	0.65*	0.59
0.4k	0.54	0.54	0.58	0.55	0.61*	0.62	0.57	0.62	0.58
0.5k	0.53	0.54	0.56	0.55	0.59	0.61	0.55	0.59	0.57
0.6k	0.55	0.53	0.55	0.54	0.57	0.58	0.54	0.57	0.59
0.8k	0.54	0.55	0.59*	0.57	0.62*	0.63*	0.59*	0.63	0.58
1k	0.69*	0.57	0.62*	0.65*	0.67*	0.74*	0.65*	0.75*	0.72*
1.25k	0.73*	0.57	0.66*	0.67*	0.71*	0.77*	0.68*	0.78*	0.78*
1.5k	0.68	0.57	0.64*	0.66*	0.67*	0.72*	0.69*	0.78*	0.71*
2k	0.69*	0.55	0.63*	0.63*	0.64*	0.68*	0.68*	0.71*	0.72
2.5k	0.64	0.56	0.62*	0.63*	0.66*	0.70*	0.69*	0.70*	0.65
3k	0.55	0.54	0.55	0.56	0.60*	0.60	0.57	0.62	0.53
4k	0.53	0.57	0.61*	0.61*	0.67*	0.69*	0.61*	0.69*	0.51
5k	0.56	0.58*	0.62*	0.62*	0.68*	0.72*	0.68*	0.75*	0.57
6k	0.53	0.58*	0.58*	0.61*	0.66*	0.67*	0.61*	0.77*	0.58
8k	0.53	0.57	0.54	0.54	0.60*	0.60	0.56	0.68*	0.56

* indicates that AROC is significantly different from 0.5 with $p < 0.05$.

Table 4.7: Area under the operating curve (AROC) for WBA against nine reference standards for various absorbance area indices

Absorbance Area Index	Reference standard								
	AABR	HFT	TEOAE	DPOAE	HFT+	HFT+	TEOAE+	HFT+	AABR+
					TEOAE	DPOAE	DPOAE	TEOAE+	TEOAE+
								DPOAE	DPOAE
0.25-0.3 kHz	0.60	0.59	0.62*	0.63*	0.68*	0.71*	0.65*	0.69*	0.70
0.4-0.8 kHz	0.50	0.54	0.56	0.54	0.60*	0.61	0.55	0.57	0.54
1 kHz	0.69*	0.57	0.62*	0.65*	0.67*	0.74*	0.65*	0.75*	0.72*
1.25-2 kHz	0.71*	0.56	0.69*	0.71*	0.70*	0.77*	0.74*	0.81*	0.74*
2.5 kHz	0.64	0.56	0.62*	0.63*	0.64*	0.68*	0.68*	0.71*	0.72
3-4 kHz	0.57	0.55	0.59*	0.66*	0.63*	0.66*	0.62*	0.58*	0.66
5-8 kHz	0.53	0.58*	0.56	0.62*	0.62*	0.72*	0.62*	0.76*	0.59

* - denotes difference was significant < 0.05

4.6 Discussion

4.6.1 Test performance of WBA

The aim of the present study was to evaluate the test performance of WBA against various reference standards to determine how WBA compares with single tests (AABR, HFT, TEOAE and DPOAE) and a battery of tests (HFT+TEOAE, HFT+DPOAE, TEOAE+DPOAE, HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE) in terms of the accuracy of predicting test outcomes in the neonatal population. As demonstrated in Tables 6 and 7, the AROC of WBA for the test battery reference standards were, in general, higher than that for single tests. The HFT+DPOAE and HFT+TEOAE+DPOAE test battery reference standards had higher test performance than that of other test batteries. WBA for HFT reference standard was significantly lower than WBA for all test battery reference standards between 1 and 2 kHz, and between 5 and 8 kHz. The WBA for the TEOAE reference standard was also significantly lower than the WBA for HFT+DPOAE and HFT+TEOAE+DPOAE reference standards between 5 and 8 kHz.

In general, the test performance of WBA against the three test battery reference standards as indicated by AROC values attaining the highest value at 1.25 kHz wherein the AROC measured against the HFT+TEOAE+DPOAE AABR+TEOAE+DPOAE reference standards was 0.78, while that against the HFT+DPOAE reference standard was 0.77. Better performance with test battery reference standards was expected because a combination of several component tests has been reported to provide a better perspective on any disease condition than any of the individual tests alone (Baughman et al. 2008; Naaktgeboren et al. 2013). The high test performance of WBA against the best possible composite reference standard (HFT+TEOAE+DPOAE) available in the present study suggests that WBA can differentiate ears with normal conductive pathway from those with a conductive condition in newborns.

In comparison, the test performance of WBA against single tests was low with AROC ranging from 0.57 to 0.73 at 1.25 kHz. The low performance of WBA against the single test reference standards may indicate that a single test per se may not accurately diagnose conductive conditions in neonates. There are at least four possible

reasons to account for this phenomenon. First, the pass criteria for a single test may not be optimal for diagnosing conductive conditions. For instance, only single peaked tympanograms were included for a pass in HFT while double peaks and multiple peaks were considered a fail. The interpretation of HFT findings has not been unanimously agreed upon. For instance, Kei et al. (2003) and Margolis et al. (2003) reported single peaked tympanograms to be indicative of normal middle ear function while Swanepoel et al. (2007) suggested that double-peaked tympanograms were also indicative of normal middle ear transmission because strong TEOAEs were obtained from these ears. Similarly, AABR is not sensitive to slight and mild conductive hearing losses (Stapells, 2011). Furthermore, the pass criteria for TEOAE of reproducibility of at least 70% and a SNR of at least 3 dB at 2, 3 and 4 kHz may not be optimal for detecting conductive conditions, especially when the pass criteria did not assess frequencies below 2 kHz where the effect of middle ear disorder may be more significant. Second, apart from AC and BC TB ABR measures, there is no gold standard for detecting conductive conditions in newborns. The tests employed in the present study are not specially designed for detecting conductive hearing losses. For example, a refer result in AABR, DPOAE and TEOAE tests may also indicate the presence of a sensorineural hearing loss. Furthermore, a pass result in AABR does not rule out slight/mild hearing losses (Stapells, 2011). Likewise, a pass in a TEOAE test cannot completely rule out middle ear dysfunction in children (Driscoll et al. 2001). Although Driscoll et al. (2001) reported these findings for six-year-old children, the results of the present study suggest that it may hold for the neonatal population as well. Third, the findings of some tests are affected by environmental and/or physiologic noise, resulting in false positive outcomes. In particular, physiologic noise in neonates could confound TEOAE and DPOAE results (Driscoll et al. 1999). The AABR test is susceptible to myogenic noise from neonates, resulting in false positive responses (Herrman et al. 1995). Fourth, the single tests could not reliably provide adequate clinical information for detecting conductive conditions. HFT findings are derived from a stimulus of a single frequency (1 kHz) which may provide limited information on the acoustic-mechanical properties of the outer and middle ear. The AABR test does not provide frequency-specific information on the function of the outer and middle ear.

The test performance for WBA against DPOAE in the present study was lower than that reported in other studies. For instance, Sanford et al. (2009) investigated test performance of WBA in terms of its ability to predict DPOAE outcomes in 455 neonate ears and reported an AROC of 0.87 for WBA. Similarly, Hunter et al. (2010) studied the test performance of WBA to predict DPOAE outcomes in 324 neonates and reported an AROC of 0.90 for WBA at 2 kHz and 0.82 for WBA at 1 kHz. In comparison, the present study found the AROC for different reference standards to range between 0.53 and 0.78. In addition, the AROC of WBA at a fixed frequency was 0.63 against TEOAE at 2 kHz and 0.63 against DPOAE at 2 kHz (Table 6).

The difference in AROC between the previous studies and the present study can be attributed to a plethora of factors including differences in equipment, test environment, sample size and participant characteristics such as ethnicity and age. In fact, the ethnicity of subjects in the present study differs from earlier WBA test performance studies due to inclusion of Aboriginal infants in the study. Previous studies have shown that Australian Aboriginal infants have a higher prevalence of middle ear dysfunction (Aithal et al. 2012; Boswell & Nienhuys 1995, 1996; Lehmann et al. 2008). Recently, Aithal et al. (In press) found that Aboriginal neonates had lower WBA than their Caucasian counterparts. Further research is needed to determine the test performance of WBA in the Aboriginal neonatal population.

The test performance of WBA may vary depending on the time of testing during the postnatal period. Due to the possible presence of vernix and/or mesenchyme in the outer and middle ear at birth, the referral rate of neonates in tests such as DPOAE, WBA and HFT may vary depending on the time of assessment (White et al. 1993; Roberts et al. 1995; Sanford et al. 2009; Hunter et al. 2010). Previous studies showed that due to transient outer/middle ear factors, the referral rate during newborn hearing screening is high in the first 24 hours of life (Hunter et al. 2010; Roberts et al. 1995; White et al. 1993). The mean age of the neonates at the time of testing in the present study was 43.7 hours while it was 25.5 hours and 29 hours with Sanford et al. (2009) and Hunter et al. (2010) studies, respectively. Sanford et al. (2009) using DPOAE as reference standard found a difference in test performance of WBA between one- and two-day-old neonates. One-day-old neonates

had an AROC of 0.87, while two-day-old neonates had an AROC of 0.74. The AROC of two-day-old neonates in Sanford et al.'s study is similar to the AROC found in the present study. In view of resolution of outer/middle ear conditions in the first few days of life, it is very important to consider the time of assessment after birth as a contributing factor in making comparisons of test performance of outer/middle ear measures in neonates.

4.6.2 Comparison of WBA across frequency regions

The WBA for all reference standards for the pass condition was within the normative range described by Aithal et al. (2013) across all of the frequencies between 0.25 and 8 kHz. Frequency distribution of WBA for the pass condition across all reference standards was in agreement with the earlier normative WBA studies in neonates (Hunter et al., 2010; Merchant et al., 2010; Sanford et al., 2009; Shahnaz, 2008). The absorbance was high between 1.25 and 2 kHz and low below 1 kHz and above 4 kHz. In contrast, the WBA in ears that failed various reference standards was relatively flat with small variations across the frequency range. With the exception of the HFT test, the WBA for all of the other reference standards was below the normative range across the entire frequency spectrum.

Median WBA for the fail condition was lower than that for the pass condition across all frequencies for all reference standards. There was a significant difference in WBA between pass and fail conditions across most of the frequencies for all reference standards except for the AABR test. This agrees with the findings of previous reports that ears with middle ear dysfunction have significantly lower absorbance compared to ears with normal middle ear function in neonates and young infants (Hunter et al., 2008, 2010; Sanford et al., 2009; Vander Werff et al., 2007; Shahnaz, 2008).

For all reference standards except HFT, AABR and AABR+TEOAE+DPOAE, the AROC for WBA in the frequency region between 1 and 2.5 kHz significantly differed from 0.5, indicating that this frequency region provided greatest discriminability between neonates who passed or failed these reference standards (Tables 6 and 7). Additionally, except for AABR, the WBA in the AAI from 1 to 2.5 kHz was significantly lower for single tests compared to test battery reference standards. This result confirms the findings of the studies that reported 1 to 2.5 kHz to

be the best frequency region to evaluate middle ear function in neonates. For example, Hunter et al. (2010) found that at 2 kHz and frequency regions involving 2 kHz (1 to 2 kHz and 1 to 4 kHz), WBA provided the greatest discriminability between the ears with a DPOAE pass or refer result. In another study, Sanford et al. (2009) found that the DPOAE refer ears had lower absorbance than DPOAE pass ears, with the best separation of WBA results between 1.4 and 2.5 kHz.

Similar to the findings of Sanford et al. (2009) and Hunter et al. (2010), the AROC values were lower at the frequencies between 0.25 and 0.8 kHz for all reference standards than at higher frequencies. Despite the low AROC values, AROC was significantly greater than 0.5 at 0.25 and or 0.3 kHz for the test battery reference standards. This finding may suggest that the test performance of WBA evaluated against these reference standards was good enough to identify conductive conditions which affect the hearing of neonates particularly at these frequencies. However, this conclusion should be interpreted with caution because WBA at low frequencies may be affected by having an inadequate probe seal during testing (Hunter et al. 2010).

Regarding the high frequencies, the AROC for WBA was significantly different from 0.5 between 4 and 6 kHz for all the reference standards except AABR and AABR+TEOAE+DPOAE tests. While this finding indicates the usefulness of WBA at these high frequencies, Aithal et al. (2013) cautioned that these frequencies may not be the best for diagnostic purposes because of the large normative range of WBA in this frequency region. Sanford et al. (2009) also found that there was more overlap in WBA above 4 kHz than 1 to 2 kHz between the neonates who passed or failed the DPOAE test. Additionally, the variation of WBA was high in the frequencies between 4 and 8 kHz in both pass and fail conditions (Figures 4.2 and 4.5). Hence, the 4 to 8 kHz range may not be useful for identification of middle ear pathology.

4.6.3 WBA measured across the reference standards

The present study also compared WBA across the reference standards under both pass and fail conditions. ANOVA results for the pass condition showed that there was no significant difference in WBA across different reference standards. An examination of the median WBA values showed similar WBA results across all reference standards (Figure 1). These median WBA results are in good agreement

with the normative WBA data obtained by Aithal et al. (2013) and Sanford et al. (2009). Increased absorbance for all references standards in the 1 to 4 kHz range compared to other frequencies was also in agreement with previous normative studies in neonates (Hunter et al., 2010; Merchant et al., 2010; Sanford et al., 2009; Shahnaz, 2008)

In comparison, the WBA measured in ears that failed the respective reference standards showed a relatively flat pattern across the entire frequency range with minor peaks and troughs from 1 to 8 kHz (Figure 4.4). The best reference standard was HFT+TEOAE+DPOAE which gave the lowest WBA values across all frequencies for ears that failed this reference standard. On the contrary, the worst reference standard was HFT which gave the highest WBA across the frequencies. It can be deduced from these results that some of the 89 ears that failed in the HFT test could have an unobstructed conductive pathway (Table 2). In other words, HFT resulted in high false positive referrals. This could be due to inclusion of ears with double or multiple peaked tympanograms for the fail condition. Swanepoel et al. (2007) consider ears with double peaked tympanograms as having normal middle ear function. Consequently, improvements in modifying the pass/fail criteria and standardizing the methods for interpreting HFT results are warranted.

Similarly, the test performance of AABR and AABR+TEOAE+DPOAE tests was lower compared to other reference standards. Since AABR is not sensitive to slight to mild conductive conditions (Stapells, 2011), many ears with middle ear dysfunction could have passed the AABR. In the present study, large numbers of ears failed HFT, TEOAE and DPOAE but passed AABR. Lesser numbers of ears that failed AABR or AABR test batteries compared to the ones that passed could have resulted in poor performance for these reference standards. Thus, AABR also resulted in high false positive referrals. Due to this reason, an adjunct test to middle ear function along with AABR screening is highly desirable.

In view of the above findings, while acknowledging the limitations of single test reference standards, the use of two or more tests as a reference standard during evaluation of test performance of WBA is recommended. Individual tests such as AABR, HFT or OAEs are not adequate to determine the middle ear status in a

screening context. This recommendation is also supported by the high test performance of WBA based on the test battery reference standards (HFT+TEOAE, HFT+DPOAE and HFT+TEOAE+DPOAE) and the low WBA values in ears that failed in these reference standards.

4.6.4 Clinical application of WBA

The finding that the test performance of WBA was high against a combination of tests compared to single tests of middle ear function suggests that WBA is a valid measure of outer and middle ear function. Due to its high accuracy, validity and brief testing time, WBA shows great promise as an adjunct test for middle ear assessment in newborn hearing screening programs. The WBA test could be very useful in prioritising neonates for further diagnostic evaluation.

In addition to screening, WBA can be used to accurately identify conductive conditions and, hence, can be employed as a diagnostic tool during the neonatal period. Moreover, during assessment of middle ear function, WBA could be used as a single clinical tool with high test performance which is as good as, if not better than, that of the test battery reference standards. In view of these properties, WBA has advantages over other tests in a clinical setting. First, objective WBA pass/fail criteria have been established using normative regions to determine middle ear status. On the contrary, there is no consensus on the criteria for pass/fail HFT or TEOAE tests. Second, WBA can be used as a single test in lieu of multiple tests such as HFT and OAE to determine the middle ear status in neonates. This can reduce testing time, especially when infants are not well settled for long periods. Third, the WBA test does not cause discomfort to infants because it can be conducted without the need to pressurize the ear canal as in HFT testing.

4.6.5 Strengths And Limitations

Earlier studies have compared the test performance of WBA against either DPOAE (Sanford et al. 2009; Hunter et al. 2010) or a combination of TEOAE and DPOAE tests (Keefe et al. 2003). This is the first study that has compared WBA against nine reference standards involving either single tests or a combination of tests in neonates.

One of the limitations of the present study is the use of a strict parallel protocol for determination of pass/fail status with test battery reference standards. This strict protocol had excluded the ears that passed one test but failed the others in a test battery reference standard. This reduced the sample size and, consequently, reduced the power of the statistical analyses employed in the present study.

Another limitation is that administration of the multiple tests using different probes might disturb the neonate, resulting in the neonate being unsettled. Calming the neonate was necessary at the expense of increasing test duration. However, if the neonate remained unsettled, the tests had to be aborted. Further research is recommended using equipment that allows all tests (e.g., DPOAE, TEOAE, HFT and WBA) to be performed using a single probe.

Furthermore, the results of the study could have been influenced by the pass/fail criteria of some tests. For instance, the pass criterion for HFT was a single positive peak with static admittance ≥ 0.2 mmho, while double or multiple peaks were considered a fail. Similarly, the TEOAE criterion of at least a 3 dB SNR in three frequency regions might be too lenient. However, we performed a post hoc analysis to compare the AROC of WBA against the TEOAE reference standard between using the present criteria and using the criteria of ≥ 3 dB at 1.5 kHz and ≥ 6 dB from 2 to 4 kHz adopted by Shahnaz (2008). Interestingly, there was no significant difference in AROC at any frequency between the two TEOAE criteria. In particular, the AROC values of WBA at 2 kHz against TEOAE using the present criteria and the Shahnaz's (2008) criteria were 0.63 and 0.62, respectively, with no significant difference between them ($p > 0.05$).

Finally, it was difficult to evaluate outer/middle ear function in neonates. AABR was performed for all infants in the present study. A pass in AABR with a fail in the other tests such as TEOAE, DPOAE and HFT may suggest a slight conductive condition. Conversely, a fail in AABR per se may suggest a significant conductive and/or sensorineural hearing loss in excess of 35-40 dB HL (Stapells 2011). Even if test battery reference standards were used to evaluate outer/middle ear function, a pass in these standards cannot definitively rule out slight conductive condition in neonates. At the very best, WBA as well as the best performing HFT+TEOAE+DPOAE

reference standard may only serve as a surrogate gold standard for evaluating outer/middle ear function in newborns.

4.6.6 Summary

The test performance of WBA against test battery reference standards was superior to that against single test reference standards. The AROC was greater for all reference standards between 1 and 4 kHz than at other frequencies, indicating the importance of measuring WBA at these frequencies for the neonatal population. In particular, the AROC reached its maximum value of 0.78 at 1.25 kHz for comparison with the HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE test battery reference standards. Due to its high performance in classifying ears with conductive loss as determined by the best performing surrogate gold standards (HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE), WBA is a desirable measure of conductive conditions in newborns. Consequently, as a valid test of conductive conditions, WBA can be used in both screening and diagnostic evaluations in neonates.

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Chapter Five: Wideband Absorbance In Australian Aboriginal And Caucasian Neonates

5.1 Background

As shown in Chapter 2, the prevalence of conductive hearing loss was twice as high in Aboriginal infants (35.19%) compared to non-Aboriginal infants (17.83%). The main cause of conductive hearing loss was otitis media. Although otitis media has been reported to begin early in infancy in Aboriginal children, systematic investigation in evaluating the function of the middle ear at birth is scarce. In recent years, wideband absorbance (WBA) has been shown to be an objective tool in the assessment of middle ear status in neonates. This study compared the status of the conductive mechanism of Aboriginal and Caucasian neonates using WBA. Results comparing WBA between Aboriginal and Caucasian neonates are presented in the Chapter Five of this thesis.

Chapter Five of this thesis, entitled, “Wideband absorbance in Australian Aboriginal and Caucasian neonates”, is based on the article accepted for publication in the *Journal of the American Academy of Audiology*. This article is inserted into this thesis with minor modifications. Only the formatting of section sub-headings and numbering of tables and figures have been modified from the original publication to match the thesis format. The referencing format of the article is retained as per the *Journal of the American Academy of Audiology* format.

Aithal, S., Kei, J., & Driscoll, C. Wideband absorbance in Australian Aboriginal and Caucasian neonates. Article accepted for publication in Special Issue of the Journal of the American Academy of Audiology

5.2 Abstract

Background Despite the high prevalence of otitis media in Australian Aboriginal infants and children, the conductive mechanism of the outer and middle ear of Aboriginal neonates remains unclear. Differences in characteristics of the conductive pathway (outer and middle ear) between Aboriginal and Caucasian neonates have not been systematically investigated using wideband acoustic immittance (WAI) measures.

Purpose The objective of this study was to compare wideband absorbance (WBA) in Australian Aboriginal and Caucasian neonates who passed or failed a screening test battery containing high frequency tympanometry and distortion product otoacoustic emissions.

Research Design A cross-sectional study design was used. The mean WBA as a function of frequency was compared between Aboriginal and non-Aboriginal neonates who passed or failed the test battery.

Study sample A total of 59 ears from 32 Aboriginal neonates (mean age = 51.9 hours, SD = 18.2, Range = 22 to 86 hours) and 281 ears from 158 Caucasian neonates (mean age = 42.4 hours, SD = 23.0, Range = 8.1 to 152 hours) who passed or failed 1000-Hz tympanometry and distortion product otoacoustic emissions were included in the study.

Data collection and analysis WBA results were analysed using descriptive statistics and t-tests with Bonferroni adjustments. An analysis of variance with repeated measures was applied to the data.

Results Aboriginal and Caucasian neonates had almost identical pass rates of 61% as determined by the test battery. Despite the apparently equal pass rates, the mean WBA of Aboriginal neonates who passed the test battery were significantly lower than that of their Caucasian counterparts at frequencies between 0.4 and 2 kHz. Mean WBA of Aboriginal neonates who failed the test battery were significantly lower than that of their Caucasian counterparts who also failed the test battery at frequencies between 1.5 and 3 kHz. Both Aboriginal and Caucasian neonates who failed the test battery had significantly lower WBA than their counterparts who passed the test battery.

Conclusion This study provided convincing evidence that Aboriginal neonates had significantly lower WBA than their Caucasian counterparts, although both groups had equal pass rates as determined by the test battery. While the two ethnic groups showed

significant differences in WBA, the factors contributing to such differences remain undetermined. Further research is warranted to determine the factors which may account for the difference in WBA between the two ethnic groups.

Key words Aboriginal, conductive pathway, middle ear, neonate, wideband absorbance

Abbreviations:

AABR – Automated auditory brainstem response

ANOVA – Analysis of variance

DPOAE – Distortion product otoacoustic emission

HFT – High frequency tympanometry

OM – Otitis media

TEOAE – Transient evoked otoacoustic emission

WAI – Wideband acoustic immittance

WBA – Wideband absorbance

WBR – Wideband reflectance

Key words Aboriginal, conductive pathway, middle ear, neonate, wideband absorbance

5.3 Introduction

Wideband acoustic immittance (WAI) has been found to be useful for detecting conductive conditions in infant ears (Keefe et al., 2003; Vander Werff et al., 2007; Hunter et al., 2008; Sanford et al., 2009; Hunter et al., 2010; Feeney and Sanford, 2012). Of the WAI measures, wideband power reflectance (WBR) has been the most frequently assessed measure in infants. Due to better test performance of WBR compared to 1000-Hz tympanometry in predicting outcomes of distortion product otoacoustic emission (DPOAE) testing, WBR is recommended to be used as an adjunct tool in newborn hearing screening programs (Sanford et al., 2009; Hunter et al., 2010).

While ear and gender differences in WBR have been well investigated in neonates (Keefe et al., 2000; Shahnaz, 2008; Hunter et al., 2010; Merchant et al., 2010), there are no studies that have investigated ethnic differences in WBR for this age group. In contrast, earlier studies with children and adults have shown significant differences in WBR across different ethnic groups. For instance, Shahnaz and Bork (2006) found significant difference in WBR between Caucasian and Chinese adults. They also found a significant interaction between ethnicity and frequency, with the Chinese group demonstrating significantly lower WBR at higher frequencies and higher WBR at lower frequencies than their Caucasian counterparts. Beers et al. (2010) studied WBR in Caucasian and Chinese children aged five to seven years. While they reported no significant main effect of ethnicity, they found a significant ethnicity by frequency interaction with the Chinese children having lower WBR values over the mid-frequency range. Apart from the ethnic differences in WBR between Caucasian and Chinese children and adults, there have been no other WBR studies on ethnic differences in other populations.

In Australia, there is a high prevalence of otitis media (OM) in Aboriginal infants (Rebgetz et al., 1989; Boswell and Nienhuys, 1995; Douglas and Powers, 1989; Foreman et al., 1992). The higher incidence of OM in Aboriginal than Caucasian infants might suggest that ethnic differences in middle ear characteristics could exist between these two groups. However, there is limited research on comparison of the function of the conductive pathway (outer and middle ear) between Aboriginal and Caucasian infants at birth. In a longitudinal study of 22 Aboriginal infants, Boswell and Nienhuys (1995) showed that 95% of these infants had OM within six to eight weeks after birth. They also reported that once middle ear disease started early in life, it became persistent despite treatment. Aithal et al. (2012) studied 211 infants (54 Aboriginal, 157 Caucasian) referred through a newborn hearing screening program and found the prevalence of conductive hearing loss to be significantly higher in Aboriginal infants (35.19 %) compared to Caucasian infants (17.8%). Prevalence of middle ear pathology was also higher in Aboriginal infants (44.4%) compared to Caucasian infants (28.7%). Aboriginal infants also showed poor resolution of conductive hearing loss over time with 66.7% of Aboriginal infants reviewed showing persistent conductive hearing loss (defined as conductive hearing

loss present during both initial diagnostic evaluation and subsequent review) compared to 17.9% of Caucasian infants.

To date, there has been only one longitudinal study of ear health in Aboriginal and Caucasian infants. Lehmann et al. (2008) monitored middle ear function in 100 Aboriginal and 180 Caucasian infants from birth to 2 years of age using transient evoked otoacoustic emissions (TEOAEs), tympanometry and otoscopic examination by an otolaryngologist. They found that TEOAEs were present in 90% (46/51) of Aboriginal and 99% (120/121) of Caucasian neonates aged less than one month. They also found that TEOAEs were present in 62% (21/34) Aboriginal and 93% (108/116) Caucasian infants aged 1-2 months. Aboriginal infants who failed TEOAEs at age 1-2 months were 2.6 times more likely to develop OM subsequently than those who passed. In comparison, a failed TEOAE outcome could not predict subsequent OM in Caucasian infants aged 1-2 months.

With the exception of the study by Aithal et al. (2012) who used a combination of high frequency (1000 Hz) tympanometry (HFT), TEOAE and auditory brainstem response (ABR) tests, all other studies have used otoscopy and/or 226-Hz tympanometry and TEOAE to compare auditory function in Aboriginal and Caucasian infants. However, both otoscopy and 226-Hz tympanometry have been found to be unreliable in evaluating middle ear function in young infants (McLennan and Webb, 1957; Jaffe et al., 1970; Cavanaugh, 1987; Holte et al., 1990; Williams et al., 1995; Mckinley et al., 1997). In order to describe the association between middle ear function and age of onset of ear diseases, it is necessary to document the status of the conductive mechanism at birth in Aboriginal infants using an efficient assessment tool. To date, there have been no studies that have investigated differences between Aboriginal and Caucasian neonates using WAI measures.

The objective of the present study was to compare wideband absorbance (WBA, one minus power reflectance) at ambient pressure between Australian Aboriginal and Caucasian neonates who passed or failed a test battery containing HFT and DPOAE screening tests. WBA is used in the present study rather than wideband power reflectance because absorbance is generally greater in normal ears than ears

with otitis media, similar to the traditional single-frequency admittance measures familiar to clinicians.

5.4 Method

5.4.1 Subjects

Aboriginal and Caucasian neonates were recruited from the Townsville Hospital in the tropical region of Queensland, Australia. Not all infants born at the Townsville Hospital were available for the study as consenting and data collection were limited to specific times of the day due to varying working rosters of research staff in the project. Ethical approval for the study was obtained from the Townsville Health Service District Institutional Ethics Committee and the University of Queensland Behavioural and Social Sciences Ethical Review Committee. Written consent was obtained from parents. All participants were born full-term with no medical complications.

A total of 195 neonates that included 32 Aboriginal (19 males, 13 females) and 163 Caucasian neonates (88 males, 75 females) were recruited for the study. Of the 195 neonates, one ear of an Aboriginal neonate and six ears of Caucasian neonates that did not pass automated auditory brainstem response (AABR) screening were excluded from the study. Furthermore WBA test could not be completed in 43 neonates (four Aboriginal, 32 Caucasian) because either the equipment was not available or the neonates were very restless. Hence, a total of 59 ears (30 right, 29 left) of Aboriginal and 281 ears (134 right, 147 left) of Caucasian neonates were included for analysis.

5.4.2 Equipment

HFT was performed with a 1000-Hz probe tone using a GN Otometrics Otoflex acoustic immittance device. Admittance was measured as the pressure was varied from +200 to -400 daPa at a rate of 400 daPa/sec. A visual system was used to classify the tympanometric results. The pass criterion was a single positively peaked tympanogram with tympanometric peak pressure occurring between 50 and -150 daPa (Kei et al., 2003; Margolis et al., 2003; Alaerts et al., 2007).

The DPOAE screen was performed using a Biologic Navigator Plus device. DPOAEs were measured in response to pairs of primary tones with F2 set at 1.5, 2, 3, 4, 6 and 8 kHz. The F2/F1 ratio was 1.2 for each primary pair. The level of F1 was 65 dB SPL and F2 was 55 dB SPL. For the purpose of screening in this study, the DPOAE findings at 1.5 and 8 kHz were not used. Hence, the pass criteria included (i) DPOAE-to-noise ratio of at least 6 dB in at least three out of four frequencies from 2 to 6 kHz (Sanford et al., 2009; Hunter et al., 2010) and (ii) DPOAE amplitude of at least -6 dB at 2, 3, 4 and 6 kHz (Sanford et al., 2009; Merchant et al., 2010).

WBA measurements were carried out using an Interacoustics Reflwin research system which consisted of a Windows-based computer, a 24-bit resolution sound card, a pressure pump and controller system contained in an acoustic immittance instrument (AT235), and custom software for stimulus generation and data acquisition. Calibration was performed every day to determine the source reflectance and incident sound pressure associated with the probe and its transducers based on acoustic measurements in two rigid walled cylindrical calibration tubes 232.3 cm and 5.6 cm in length (Keefe and Simmons, 2003).

WBA was measured at ambient pressure by recording the acoustic response to clicks presented to the neonate's ear. Absorbance was calculated by averaging responses from a total of 16 clicks. The WBA response consisted of 16 data points (at 1/3 octave frequencies from 0.25 to 8 kHz). During testing, high absorbance at frequencies below 1 kHz indicated a possible probe leak. When this occurred, testing was aborted and resumed when a hermetic seal was obtained. A visual prompt also alerted the tester if the noise level was high.

5.4.3 Procedure

All measurements were performed by an experienced audiologist in a quiet room in the maternity unit of the hospital. The mean ambient noise level in the testing room was 35.7 dB A (SD = 2.1, Range = 31.1 to 43.8 dB A). Neonates were usually tested after feeding while in natural sleep or in an awake but quiet state. The most accessible ear was tested first. All the tests were completed on one ear and the second ear was tested if the neonate was well settled and there was adequate time for testing. AABR screening was done using click stimuli at the level of 35 dBnHL. Only the ears

that passed the AABR screen were included in the study. A pass in AABR suggested normal hearing and, thus, a refer for either or both of the DPOAE and HFT screening tests was suggestive of a middle ear dysfunction not detected by the AABR. While passing AABR indicates global normal auditory function, AABR is not sensitive to subtle middle ear and cochlear conditions (Mazlan et al., 2009; Kei, 2012). Following the AABR screen, the audiologist performed HFT, DPOAE and WBA tests, in no particular order.

Driscoll et al. (2000) recommended that tympanometry should be used along with otoacoustic emissions (OAEs) if identifying middle ear disorders is the goal of screening. Therefore, a test battery of HFT and DPOAE was used to screen for conductive conditions in the present study. Although tympanometry and DPOAE screening tests are useful for assessing the function of the conductive pathway, they could not be regarded as a gold standard for middle ear function. In the present study, in the presence of a pass in AABR, a pass in both tympanometry and DPOAE tests in the test battery was considered to indicate an efficient conductive pathway, while a fail in both tests was indicative of an inefficient conductive pathway.

5.4.4 Statistical analysis

Significance of difference between the proportion of Aboriginal and Caucasian neonates with normal or abnormal results on tests of outer/middle ear function was analysed using a two-proportion Z-test with a significance level of 0.05.

A mixed model analysis of variance (ANOVA) was used to analyse data obtained from Aboriginal and Caucasian neonates who passed or failed the HFT and DPOAE screening test battery. The Greenhouse and Geisser G-G approach (1959) was used to compensate for the violation of compound symmetry and sphericity. An alpha level of 0.05 was used for all analyses. Data from neonates in both groups who passed the test battery were analysed using three between-group factors: gender (male versus female), ear (right versus left) and ethnicity (Aboriginal versus Caucasian) and one within-group factor (frequency). Data from neonates who failed the test battery were analysed using ethnicity as between-group factor and frequency as within-group factor. Post hoc analyses were performed using multiple pairwise comparison tests with Bonferroni adjustment to determine the frequencies at which significant

differences existed between Aboriginal and Caucasian neonates who passed and those who failed the test battery.

5.5 Results

5.5.1 Test battery pass/fail.

Table 5.1 illustrates the proportion of ears of Aboriginal and Caucasian neonates who passed or failed the HFT and DPOAE screening test battery. A total of 208 ears (36 Aboriginal, 172 Caucasian) passed the test battery and 96 ears (19 Aboriginal and 77 Caucasian) failed the test battery. Approximately 61% of neonates in both ethnic groups passed the test battery. Further analysis using a Z-test showed no significant difference in the proportions of Aboriginal and Caucasian ears that passed or failed the test battery. About 6% of Aboriginal ears and 11 % of Caucasian ears did not have results for all of the three tests (HFT, DPOAE and/or WBA) as the neonates were unsettled during testing.

Table 5.1: Results of a Z test for evaluating the significant difference in proportions between neonate ears that passed or failed in a test battery of HFT and DPOAE screening tests

	Aboriginal n (%)	Caucasian n (%)	Z value	Significance p
Pass	36 (61.02%)	172 (61.21%)	-0.028	0.98
Fail	19 (32.20%)	77 (27.40%)	0.745	0.46
Incomplete data	4 (6.78%)	32 (11.39%)		

5.5.2 WBA in neonates who passed or failed the test battery.

5.5.2.1 WBA in Aboriginal and Caucasian neonates who passed the HFT and DPOAE screening test battery

The demographic details of Aboriginal and Caucasian neonates who passed the test battery are shown in Table 5.2. The results of t-tests showed no significant differences in gestational age, age at time of testing and birth weight between Aboriginal and Caucasian neonates.

Table 5.2: Details of Aboriginal and Caucasian neonates who passed the test battery of HFT and DPOAE tests. The results of a t-test showed no significant difference in gestational age, age at time of testing and birth weight between Aboriginal and Caucasian neonates.

	Aboriginal	Caucasian	Significance of difference (p)
Number of subjects	23	113	
Males	15	57	
Females	8	56	
Number of ears	36	172	
Right	17	86	
Left	19	86	
Gestational age (weeks)			
Mean	39.22	39.20	0.53
SD	1.28	1.16	
Range	36-41	36-42	
Age at time of testing (hours)			
Mean	51.90	43.14	0.98
SD	18.17	20.49	
Range	22.10-86.16	9.02-116.5	
Birth weight (grams)			
Mean	3368.70	3529.91	0.07
SD	496.87	440.87	
Range	2200-4260	2290-4640	

Table 5.3 shows the results of an ANOVA to evaluate the effect of ear, gender and frequency on WBA for neonates who passed the test battery. One ear per infant was chosen and, when both ears passed the test battery, right or left ear was chosen randomly. A total of 23 Aboriginal and 113 Caucasian neonates were included in this ANOVA analysis. While the main effect for frequency was significant, the effects of ear and gender and their interactions were not significant for either the Aboriginal or Caucasian group. In view of insignificant ear and gender effects, the WBA data were pooled across ears and genders for both ethnic groups.

Table 5.3: ANOVA results of WBA obtained from Aboriginal (n=23) and Caucasian (n=113) neonates with a pass in the test battery of HFT and DPOAE.

		F value	df	P value	Observed Power
Aboriginal	Ear	2.79	1	0.11	0.35
	Gender	2.75	1	0.11	0.35
	Ear x Gender	1.10	1	0.30	0.17
	Error	4.07	19	0.21	
	Frequency	16.87	4	0.00*	1.00
	Ear x Frequency	0.75	4	0.54	0.22
	Frequency x Gender	0.80	4	0.52	0.23
	Ear x Gender x Frequency	0.98	4	0.42	0.28
	Error	4.583	67	0.07	
Caucasian	Ear	1.52	1	0.22	0.23
	Gender	5.60	1	0.02	0.65
	Ear x Gender	1.82	1	0.18	0.27
	Error	18.69	109	0.17	
	Frequency	75.12	4	0.00*	1.00
	Ear x Frequency	0.70	4	0.61	0.24
	Frequency x Gender	0.65	4	0.65	0.23
	Ear x Gender x Frequency	1.40	4	0.23	0.47
	Error	28.220	495	0.06	

Note: * indicates significant difference at an alpha level of <.05

Table 5.4 illustrates the various WBA percentile values (0, 10, 50, 90 and 100) for both Aboriginal (n= 36 ears) and Caucasian (n= 172 ears) neonates with a pass in the HFT and DPOAE screening test battery. Normative range for WBA was determined as the region between 10th and 90th percentiles (Aithal et al., 2012). Figure 5.1 illustrates the median WBA and normative region across 16 one-third octave frequencies from 0.25 to 8 kHz for Aboriginal and Caucasian neonates. The median WBA in Aboriginal neonates was lower than that of Caucasian neonates at all frequencies except 0.3, 3 and 4 kHz with an average magnitude of difference of 0.06. At the 10th percentile, the WBA of Aboriginal neonates was lower than that of Caucasian neonates at all frequencies except 0.25 kHz. At the 90th percentile, the WBA was similar for both groups across all frequencies except at 0.4 and 0.5 kHz where Caucasian neonates had slightly higher WBA than Aboriginal neonates.

Table 5.4: Absorbance values for various percentiles (0, 10, 50, 90 and 100) for Aboriginal (36 ears pass, 19 ears fail) and Caucasian (172 ears pass, 77 ears fail) neonates who passed or failed the test battery

Frequency (kHz)	Test battery	Percentiles									
		Aboriginal					Caucasian				
		0	10	50	90	100	0	10	50	90	100
0.25	Pass	0.17	0.32	0.51	0.67	0.73	0.00	0.30	0.53	0.66	0.76
	Fail	0.00	0.00	0.29	0.66	0.76	0.00	0.08	0.44	0.63	0.79
0.30	Pass	0.10	0.30	0.49	0.64	0.74	0.00	0.35	0.49	0.63	0.75
	Fail	0.00	0.00	0.40	0.67	0.80	0.00	0.12	0.46	0.61	0.76
0.4	Pass	0.05	0.24	0.38	0.53	0.69	0.00	0.31	0.43	0.58	0.77
	Fail	0.00	0.00	0.37	0.58	0.76	0.00	0.18	0.40	0.56	0.67
0.5	Pass	0.06	0.19	0.36	0.52	0.59	0.00	0.27	0.41	0.56	0.73
	Fail	0.00	0.00	0.36	0.52	0.69	0.00	0.22	0.37	0.56	0.64
0.6	Pass	0.11	0.23	0.39	0.56	0.60	0.02	0.31	0.45	0.58	0.71
	Fail	0.00	0.04	0.37	0.57	0.59	0.00	0.23	0.39	0.57	0.67
0.8	Pass	0.16	0.29	0.44	0.64	0.71	0.21	0.37	0.50	0.66	0.88
	Fail	0.00	0.05	0.38	0.56	0.69	0.00	0.23	0.45	0.63	0.74
1	Pass	0.20	0.30	0.60	0.79	0.92	0.22	0.40	0.64	0.81	0.93
	Fail	0.00	0.02	0.45	0.76	0.80	0.00	0.22	0.52	0.76	0.92
1.25	Pass	0.18	0.37	0.65	0.88	0.94	0.19	0.50	0.74	0.91	0.96
	Fail	0.00	0.00	0.41	0.82	0.83	0.00	0.10	0.59	0.86	0.95
1.5	Pass	0.15	0.36	0.68	0.91	0.93	0.29	0.51	0.77	0.90	0.99
	Fail	0.00	0.00	0.36	0.89	0.90	0.00	0.17	0.67	0.91	0.98
2	Pass	0.21	0.36	0.63	0.87	0.96	0.32	0.45	0.72	0.89	0.99
	Fail	0.00	0.07	0.40	0.79	0.84	0.03	0.21	0.60	0.88	0.95
2.5	Pass	0.07	0.22	0.53	0.76	0.81	0.06	0.28	0.56	0.78	0.90
	Fail	0.00	0.00	0.30	0.61	0.68	0.00	0.08	0.49	0.76	0.92
3	Pass	0.00	0.20	0.52	0.68	0.72	0.00	0.25	0.50	0.69	0.86
	Fail	0.00	0.00	0.26	0.57	0.58	0.00	0.07	0.45	0.71	0.82
4	Pass	0.00	0.02	0.46	0.66	0.81	0.00	0.22	0.45	0.67	0.90
	Fail	0.00	0.00	0.24	0.66	0.67	0.00	0.02	0.39	0.66	0.88
5	Pass	0.00	0.14	0.55	0.85	0.87	0.00	0.31	0.61	0.84	0.94
	Fail	0.00	0.00	0.48	0.86	0.94	0.00	0.01	0.46	0.77	0.91
6	Pass	0.10	0.30	0.67	0.96	0.99	0.00	0.35	0.75	0.92	0.97
	Fail	0.00	0.00	0.62	0.95	0.97	0.00	0.11	0.50	0.87	0.96
8	Pass	0.00	0.16	0.56	0.93	0.98	0.00	0.23	0.65	0.95	0.99
	Fail	0.00	0.00	0.45	0.81	0.87	0.00	0.09	0.56	0.92	0.99

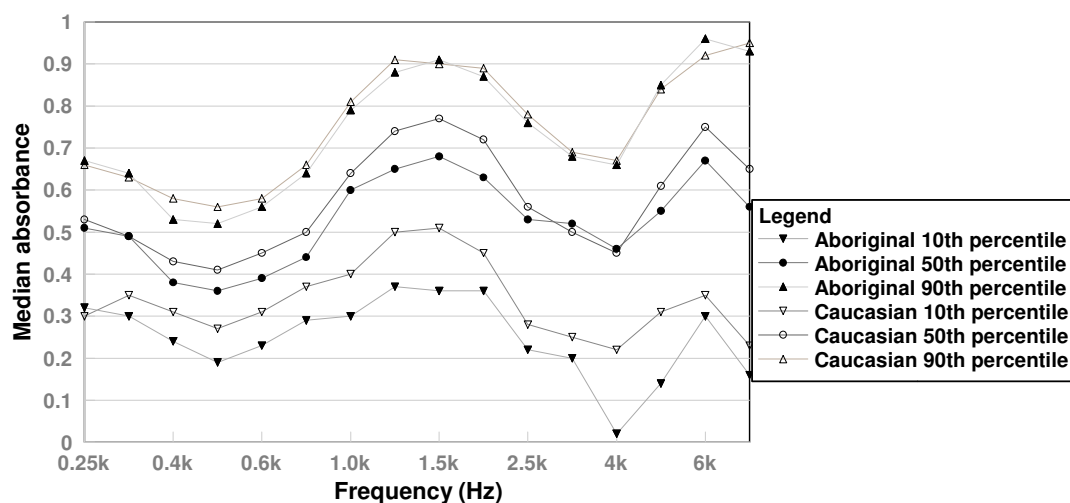


Figure 5.1: Median and normative range of WBA in Aboriginal and Caucasian ears that passed the test battery containing HFT and DPOAE

To compare WBA between the two ethnic groups, a repeated measure ANOVA was performed with ethnicity as between-subject factor and frequency (16 levels) as a within-subject factor. All the ears that passed the test battery (36 Aboriginal, 172 Caucasian) were included in the analysis. There were significant main effects for ethnicity [$F(1, 206) = 4.380$, $p = 0.04$, observed power = 0.55] and frequency [$F(5, 945) = 77.70$, $p = 0.00$, observed power = 1.00]. The Ethnicity x Frequency interaction [$F(5, 945) = 0.764$, $p = 0.57$, observed power = 0.27] was not significant. *Post hoc* analysis with Bonferroni correction was performed at each of the 16 one-third octave frequencies to analyse the effect of ethnicity. Columns 1 of Table 5.5 show the frequencies between the two groups for neonates who passed the hearing screening test battery. The WBA was significantly different between Aboriginal and Caucasian neonates from 0.4 to 2 kHz.

5.5.2.2 WBA in Aboriginal and Caucasian neonates who failed the HFT and DPOAE screening test battery

A total of 19 ears of Aboriginal neonates and 77 ears of Caucasian neonates who did not pass either one or both the tests in the screening test battery were considered for analysis and determination of various percentile measures (Table 5.4). The WBA of Aboriginal and Caucasian neonates with a fail in the test battery were compared with each other as well as with their respective counterparts who passed the

test battery. As illustrated in Figure 5.2 and column 2 of Table 5.5, the median WBA of Aboriginal neonates who failed the test battery was lower than that of the Caucasian neonates who failed the test battery. The median WBA in Aboriginal neonates was lower than that of Caucasian neonates at all frequencies except 5 and 6 kHz with an average magnitude of difference of 0.12. Difference between median WBA between Aboriginal and Caucasian neonates was highest (0.21) between 1.25 and 4 kHz. Furthermore, both Aboriginal and Caucasian neonates who failed the test battery had lower WBA across most frequencies than their counterparts who passed the test battery (columns 3 and 4 of Table 5.5).

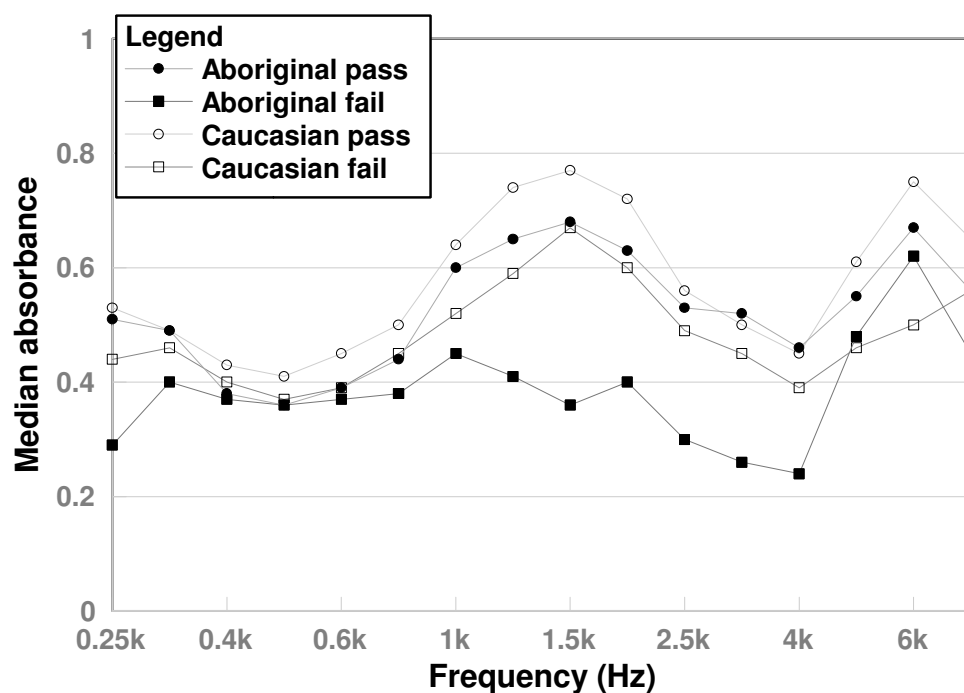


Figure 5.2: Comparison of median WBA in Aboriginal and Caucasian ears that passed or failed a test battery of HFT and DPOAE

Table 5.5: Significance of difference in WBA between Aboriginal and Caucasian neonates with a pass or fail in the test battery of HFT and DPOAEs (post-hoc ANOVA with Bonferroni correction)

Frequency (kHz)	Significance of difference in WBA between Aboriginal and Caucasian neonates who passed the test battery		Significance of difference in WBA between Aboriginal and Caucasian neonates who failed the test battery		Significance of difference in WBA between Aboriginal neonates who passed or failed the test battery		Significance of difference in WBA between Caucasian neonates who passed or failed the test battery	
	t	Significance (p)	t	Significance (p)	T	Significance (p)	t	Significance (p)
0.25	-0.17	0.86	-1.36	0.18	3.25	0.02*	4.80	0.00*
0.30	-1.25	0.21	-1.51	0.13	2.33	0.02*	3.78	0.01*
0.40	-2.25	0.03*	-0.94	0.35	0.70	0.49	2.48	0.01*
0.50	-2.38	0.02*	-0.85	0.40	0.49	0.63	2.39	0.02*
0.60	-2.45	0.01*	-1.43	0.16	1.23	0.22	3.08	0.00*
0.80	-2.43	0.01*	-1.60	0.11	1.95	0.05	4.40	0.00*
1	-2.18	0.03*	-1.91	0.05	3.02	0.00*	5.64	0.00*
1.25	-2.61	0.01*	-1.91	0.05	3.55	0.00*	6.32	0.00*
1.5	-2.36	0.02*	-2.18	0.03*	3.60	0.00*	5.46	0.00*
2	-2.27	0.02*	-2.69	0.01*	3.70	0.00*	4.22	0.00*
2.5	-1.08	0.28	-2.66	0.01*	4.11	0.00*	3.66	0.00*
3	-0.32	0.75	-2.49	0.02*	3.45	0.00*	2.42	0.02*
4	-0.84	0.40	-1.38	0.10	2.15	0.04*	3.14	0.00*
5	-0.59	0.56	0.35	0.99	1.30	0.20	4.97	0.00*
6	-0.79	0.43	0.43	0.88	1.23	0.22	4.89	0.00*
8	0.97	0.33	-1.05	0.32	1.50	0.14	2.26	0.02*

Note: * indicates significant difference at an alpha level of <.05

5.5.2.3 Effects of ethnicity and test battery outcome

To compare WBA between the two groups of neonates that failed the test battery, an ANOVA was performed with ethnicity (Aboriginal vs Caucasian) as between subject factor and frequency as within subject factor. There were no significant main effects for ethnicity [$F(1, 53) = 3.018, p > 0.05$]. The main effect of frequency [$F(4, 405) = 12.91, p = 0.00$, observed power = 1.00] and interaction between frequency and ethnicity [$F(4, 405) = 2.91, p = 0.02$, observed power = 0.80] were significant. A further ANOVA to analyse the effects of test battery outcome (pass or fail) on WBA in Aboriginal neonates revealed a significant effect for test battery outcome [$F(1, 53) = 8.63, p = 0.00$, observed power = 0.82], and frequency

[F(4, 256) = 17.32, $p = 0.00$, observed power = 1.00]. There was a significant interaction between test battery outcome and frequency [F(4, 256) = 3.65, $p = 0.00$, observed power = 0.98]. Similarly, for Caucasian neonates, there was a significant main effect for test battery outcome [F(1, 247) = 30.46, $p = 0.00$, observed power = 0.98], and frequency [F(4, 1154) = 97.89, $p = 0.00$, observed power = 1.00]. There was also a significant interaction between test battery outcome and frequency [F(4, 1154) = 5.76, $p = 0.00$, observed power = 0.99].

Figures 5.3 and 5.4 illustrate group means for the Aboriginal and Caucasian neonates who passed or failed the test battery, respectively. The vertical bars denote \pm one standard error of the mean (SEM). Columns 3 and 4 of Table 5.5 illustrate the results of *post hoc* analysis using Bonferroni correction factor to analyse the mean differences between neonates who passed or failed the test battery. As illustrated in Table 5.5, the means for WBA of Aboriginal neonates who passed the test battery were significantly lower at frequencies between 0.4 and 2 kHz when compared with their Caucasian counterparts who passed the test battery. Similarly, the means for WBA between Aboriginal and Caucasian neonates who failed the test battery were significantly lower at frequencies between 1.5 and 3 kHz. Furthermore, means for WBA of Aboriginal neonates with a pass were significantly higher between 0.2 and 0.3 kHz and between 0.8 and 4 kHz when compared with the Aboriginal neonates with a fail in the test battery. In comparison, the mean WBA values of Caucasian neonates with a pass were significantly higher than those with a fail in the test battery at all frequencies.

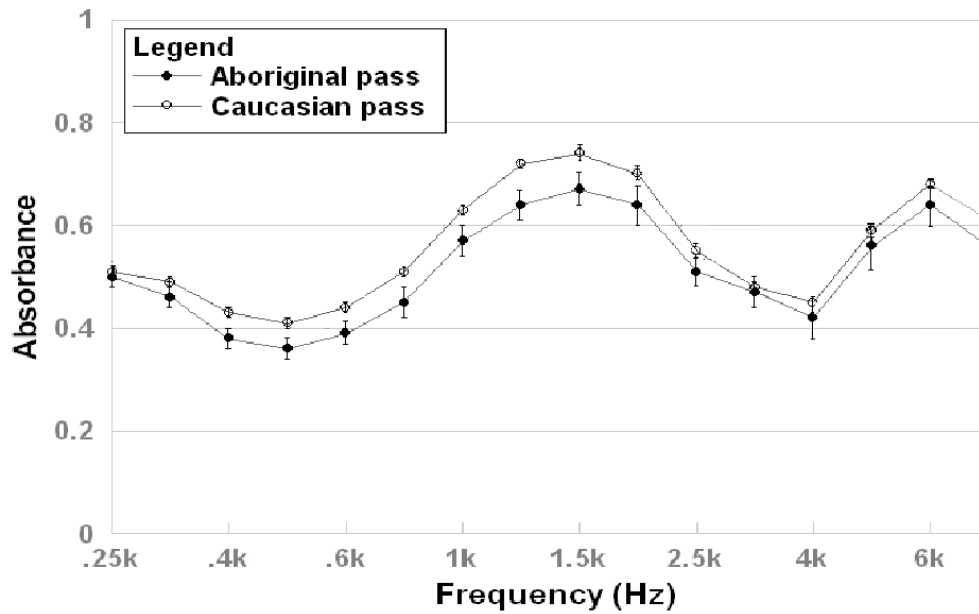


Figure 5.3: Mean WBA for Aboriginal (n = 36) and Caucasian (n = 172) ears that passed the test battery. Vertical bars denote Mean \pm 1SEM.

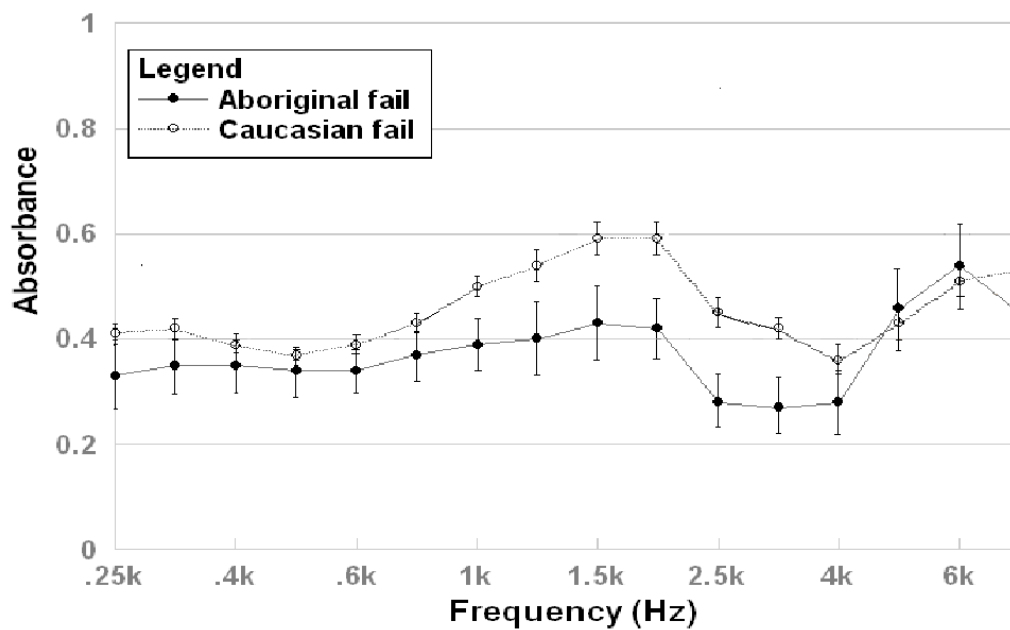


Figure 5.4: Mean WBA for Aboriginal (n = 19) and Caucasian (n = 77) ears that failed the test battery. Vertical bars denote Mean \pm 1SEM.

5.6 Discussion

The present study evaluated the function of the conductive auditory pathway in Australian Aboriginal and Caucasian neonates. The results revealed no significant difference in the proportion of Aboriginal (61%) and Caucasian (61%) neonates who passed a test battery containing HFT and DPOAE screening tests. This contrasts with the findings of earlier studies that have reported a significant difference in the pass rates between these two groups. For instance, using a combination of otoscopy and 226-Hz tympanometry tests with 1- to 14-day-old infants, Boswell and Nienhuys (1995) reported that 86% of Aboriginal and 61% of Caucasian neonates passed the two tests. Similarly, Lehman et al. (2008) found a significant difference in pass rates between Aboriginal (90%) and Caucasian (99%) neonates using TEOAEs.

Differences in pass rates can be attributed to methodological differences across studies. First, the age of neonates was different across the studies. The mean age of the Aboriginal neonates in the present study was 51.9 hours, while the infants in the Boswell and Nienhuys (1995) and Lehmann et al. (2008) were older (up to three weeks of age). The difference in age of participants among studies would account, at least partly, for the difference in pass rates given that pass rates improve with time for the first few days of life due to the clearing of external and middle ear fluids (Sanford et al., 2009; Hunter et al., 2010).

Second, Boswell and Nienhuys (1995) used a combination of otoscopy and 226-Hz tympanometry and Lehmann et al. (2008) used TEOAEs, while the present study employed a combination of HFT and DPOAE tests. Neither otoscopy nor 226-Hz tympanometry are recommended for use with neonates in view of inaccurate test outcomes (Sprague et al., 1985; Doyle et al., 1997; Rhodes et al., 1999; Margolis and Hunter, 2000). Additionally, the use of a single test such as HFT, TEOAE or DPOAE alone to determine outer/middle ear function has been found to be less than ideal in assessing the outer/middle ear conditions (Kei et al., 2003; Swanepoel et al., 2007; Shahnaz, 2008; Sanford et al., 2009; Hunter et al., 2010; Aithal et al., 2013). Instead, a test battery approach with appropriate measures for cochlear and middle ear function may provide more clinical information for identifying outer/middle ear conditions than that provided by a single measure (Mazlan and Kei, 2012; Aithal et al., 2013).

5.6.1 WBA in neonates who passed or failed the screening test battery

5.6.1.1 WBA in neonates who passed the HFT and DPOAE screening test battery

Aboriginal neonates who passed the test battery had significantly lower WBA than their Caucasian counterparts, especially between 0.4 and 2 kHz, the frequencies important in the determination of the status of the outer and middle ear (see Table 5.5 and Figure 5.3). This low energy absorbance into the middle ear of Aboriginal neonates in this study suggests that these neonates had more significant outer/middle ear conditions than Caucasian neonates. The Aboriginal neonates may have outer/middle ear disorders that are not detected when they are assessed shortly after birth using HFT and DPOAE screening tests, but identified by the WBA test. This has implications for the proportion of Aboriginal and Caucasian neonates who passed or failed the test battery. It is likely that a greater proportion of Aboriginal neonates would have failed the screening if a more sensitive test, such as WBA was included in the test battery. Although addition of WBA to the HFT and DPOAE test battery would still not be a gold standard, it would likely be more accurate than the HFT and DPOAE test battery. Further research could consider using a large sample size to evaluate the use of WBA during neonatal hearing screening in Aboriginal and Caucasian infants.

Shahnaz and Bork (2006) attributed WBA differences between two ethnic groups (Caucasian and Chinese) to differences in body size. An increase in body size in animal models is associated with an increase in the size of their middle ear structures, such as increased ossicle and footplate size and increased tympanic membrane area (Werner et al., 1998; Saunders et al., 2000; Werner and Igic, 2002). Additionally, Voss et al. (2008) studied normal cadaver ears and demonstrated that at frequencies below 2000 Hz, large increases in middle ear cavity volume systematically reduce the energy reflectance, with more variable changes above 2000 Hz. Relkin (1988) postulated that this increased middle ear volume could decrease the stiffness of the air in this space. Nevertheless, it is not known whether increasing body size in humans is related to increase in the volume of the external and middle ear (Shahnaz and Bork, 2006). It is also unclear how differences in the mass of the conductive mechanism relate to differences in WBR (Beers et al., 2010). The present study did not find a significant difference in birth weight (which is assumed to be

proportional to body size) between the two groups. Nevertheless, birth weight approached significance levels ($p = 0.07$), with Aboriginal neonates having smaller birth weight than Caucasian neonates, suggesting that there could be a possible link between birth weight and WBA. Further investigation using a larger sample size is recommended to explore the association between WBA and body weight.

Another possible explanation for the ethnic differences in WBA might be related to possible dissimilarities in anatomical structure and physiological function of the auditory system between the two ethnic groups, given that the Aboriginal neonates had lower WBA than their Caucasian counterparts irrespective of their outer/middle ear status as determined by the test battery. However, this hypothesized difference in anatomical and physiological characteristics between Aboriginal and Caucasian neonates has not been addressed in the literature to date.

The present study did not find any ear or gender effects for either Aboriginal or Caucasian neonates who passed the test battery. This is consistent with the findings of Hunter et al. (2008) in neonates and toddlers. Beers et al. (2010) also found no ear or gender effects in children. Although Keefe et al. (2000) found ear and gender differences in WBR in neonates, their study did not include comparative measures of outer and middle ear function, such as HFT or TEOAE.

In light of the WBA differences between Aboriginal and Caucasian neonates despite a pass in the test battery, further research is warranted to investigate the factors that may account for these differences. For instance, research using pressurised WBA might contribute to a better understanding of the physiological differences in outer/middle ear characteristics between Aboriginal and Caucasian neonates, such as comparisons of WBA at peak tympanometric pressure. Furthermore, if absorbance is influenced by body size, further research could consider including measures of body size such as birth weight, height and head circumference as possible influential factors.

5.6.1.2 WBA in neonates who failed the HFT and DPOAE screening test battery

Aboriginal and Caucasian neonates with a fail in the test battery had significantly lower WBA than their counterparts who passed. The WBA was significantly different between 0.8 and 4 kHz in the Aboriginal group and across the entire frequency region in the Caucasian group (Table 5.5). Hunter et al (2010) reported that regions involving 2kHz, particularly 1 to 2 kHz, 1 to 4 kHz and 2kHz, provide the greatest discriminability between neonates with a pass or refer during newborn screening using DPOAE. Similarly, Sanford et al (2009) reported that the best separation of neonates with a pass or refer in DPOAE screening was achieved from 1 to 2 kHz.

The finding that neonates with a fail in test battery had lower absorbance is consistent with findings of earlier studies that have shown higher reflectance (therefore, reduced absorbance) values in children with OM and conductive hearing loss (Keefe and Levi, 1996; Hunter and Margolis, 1997; Piskorski et al., 1999; Hunter et al., 2008; Sanford et al., 2009; Keefe et al., 2012). Congenital middle ear effusion, a commonly reported condition in neonates (Orlando and Prieve, 1998; Gorga et al., 2001; Boone et al., 2005) increases the mass and stiffness of the middle ear system. The measured increase in WBR in the mid to high frequency range (1 to 6 kHz) may be a direct result of the increased mass load on the middle ear system due to middle ear effusion (Beers et al., 2010; Hunter et al., 2010; Shahnaz, 2010).

Aboriginal neonates who failed the test battery in the present study had significantly lower WBA in the frequencies between 1.5 and 3 kHz compared to their Caucasian counterparts who failed the test battery (see Table 5.5 and Figure 5.4). In this frequency range, WBA ranged from 0.26 to 0.40 in Aboriginal neonates and from 0.45 to 0.67 in Caucasian neonates. This suggests that the Aboriginal neonates have more significant outer/middle ear conditions than Caucasian neonates. To date, there are no studies that have investigated anatomical and physiological differences in the outer/middle ear between these two groups of neonates. Further research using radiological evidence in infants undergoing cochlear implant surgery or investigations of head and neck as part of other investigations as well as temporal bone studies of neonates would assist in determining if a difference in the volume of the middle ear cavity is associated with difference in stiffness between Aboriginal and Caucasian neonates across frequencies that are important for speech perception (1 to 4 kHz).

Studies have shown that newborns with persistent middle ear effusion are more likely to develop chronic OM, compared with newborns without persistent middle ear effusion (Jaffe et al., 1970; Doyle et al., 2004; Pereira et al., 2010). Lehmann et al. (2008) found that Aboriginal infants who failed TEOAEs at age 1 to 2 months were 2.6 times more likely to develop OM subsequently than those who passed. In comparison, a failed TEOAE outcome did not predict subsequent OM in non-Aboriginal infants aged 1 to 2 months. Future research could follow the Aboriginal and Caucasian neonates with lower absorbance up to two years of age to see if they went on to develop OM.

5.6.2 Strengths and limitations of the study

This is the first study to compare WBA between Aboriginal and Caucasian neonates who passed or failed a test battery. Since WBA provides detailed information about the efficiency of sound conduction in the auditory system, this study holds relevance for investigating the anatomical and physiological differences in the outer/middle ear between Aboriginal and Caucasian neonates.

Although the proportion of ears that passed the test battery was the same for both groups, this finding may not be confidently generalized in view of the small sample size. The proportion of Aboriginal neonates who failed the test battery was slightly larger compared to that of Caucasian neonates. A larger sample is required to determine WBA test performance in both groups and to draw conclusions regarding ethnic differences in outer/middle ear function.

While testing was often done when a neonate was well settled, the use of multiple tests such as HFT, DPOAE and WBA required a lengthy testing time. Insertion and adjustment of various probe tips might have disturbed the neonate. Testing had to be discontinued for some neonates who became unsettled, resulting in a small number of neonates included in the study. Further modification of the equipment could incorporate a single probe to perform multiple tests to minimise this problem.

5.6.3 Summary and conclusions

The present study showed that Aboriginal and Caucasian neonates had almost identical pass rates of 61% as determined by a test battery containing HFT and DPOAE screening tests. However, the Aboriginal neonates who either passed or failed the HFT and DPOAE test battery had significantly lower WBA than their Caucasian counterparts, suggesting that Aboriginal neonates are more likely to have abnormal outer/middle ear status than Caucasian neonates. These findings may have two clinical implications. First, WBA appears to be more sensitive than HFT and DPOAE screening tests in the identification of inefficient conductive pathways in neonates. Second, there may be subtle differences in anatomical and physiological characteristics of the outer and middle ear which resulted in differences in WBA between Aboriginal and Caucasian neonates. However, the causal factors for the ethnic differences have not been identified. Further research is warranted to determine the factors which may account for the difference in WBA between the two ethnic groups. Given that Aboriginal infants are more likely to develop OM later in life than their non-Aboriginal counterparts (Lehmann et al., 2008), further research should consider measuring WBA in Aboriginal neonates from birth to two years of age using a longitudinal design. This longitudinal study may determine whether the Aboriginal neonates with outer and middle ear dysfunction during the first few days of life are prone to OM later in life. Future research may also consider evaluating ethnic differences in the relative risk of OM in infants during the first two years of life.

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Chapter Six: Wideband Absorbance In Young Infants (0-6 Months): A Cross-sectional Study

6.1 Background

The outer and middle ears undergo significant developmental changes during the first few months of life. These rapid developmental changes in early infancy could have an effect on the acoustic properties of the ear. While the standard audiological tests of middle ear function used with older children and adults are limited in their ability to determine the status of conductive mechanism in young infants, wideband absorbance (WBA) has been proposed as an emerging tool to assess outer/middle ear in this population. Thus far, the results of previous studies have been equivocal regarding the changes in WBA with increasing age especially in the first 12 months of life (Kei et al., 2013). Due to significant developmental changes during early infancy, there is a need to include infants at various age intervals in order to more closely describe the developmental effects on the outer and middle ear (Sanford and Feeney, 2008).

Results of the study comparing WBA across age groups are presented in Chapter six of this thesis. This chapter is based on the article accepted for publication in the *Journal of the American Academy of Audiology*. This article is inserted into this thesis with minor modifications. Only the formatting of section sub-headings and numbering of tables and figures have been modified from the original publication to match the thesis format. The referencing format of the article is retained as per the *Journal of the American Academy of Audiology* format.

Aithal, S., Kei, J. & Driscoll, C. Wideband Absorbance in young infants (0-6 Months): A cross-sectional study. Article accepted for publication in special supplement of Journal of the American Academy of Audiology.

6.2 Abstract

Background: Wideband acoustic immittance (WAI) studies on infants have shown changes in WAI measures with age. These changes are attributed, at least partly, to developmental effects. However, the developmental effects of young infants (0-6 months) on WAI have not been systematically investigated.

Purpose: The objective of this study was to compare wideband absorbance (WBA) in healthy neonates and infants aged 1-, 2-, 4- and 6-months.

Research Design: This was a prospective, cross-sectional study. All participants were assessed using 1-kHz tympanometry, distortion product otoacoustic emission and WBA tests.

Study Sample: Participants included 35 newborns (35 ears), 16 one-month-old (29 ears), 16 two-month-old (29 ears), 15 four-month-old (28 ears) and 14 six-month-old infants (27 ears). For each participant, the ears that passed both high frequency (1-kHz) tympanometry and distortion product otoacoustic emission tests were included for analysis.

Data collection and analysis: WBA was recorded at ambient pressure conditions and the response consisted of 16 data points at one-third octave frequencies from 0.25 to 8 kHz. A mixed model analysis of variance (ANOVA) was applied to the data in each age group to evaluate the effects of gender, ear and frequency on WBA. WBA was compared between various age groups. In addition, a separate mixed model ANOVA was applied to WBA data and post hoc analyses using Bonferroni correction were performed at each of the 16 one-third-octave frequencies across age groups to examine the effect of age on WBA.

Results: For all age groups, WBA was highest between 1.5 and 5 kHz and lowest at frequencies below 1.5 kHz and above 5 kHz. A developmental trend was evident with both the 0- and 6-month-old infants being significantly different to other age groups at most of the frequencies. The WBA results exhibited a multi-peaked pattern for infants aged 0 to 2 months, whereas a single broad peaked pattern for 4- and 6-month-old infants was observed. The difference in WBA between 0- and 6-month-old infants was statistically significant across most frequencies. In contrast, the WBA results for 1- and 2-month-old infants were comparable. There were no significant gender or ear effects on WBA for all age groups.

Conclusions: Developmental effects of WBA were evident for infants during the first 6 months of life. The WBA data can be used as a reference for detecting disorders in the sound conductive pathways (outer and middle ear) in young infants. Further development of age-specific normative WBA data in young infants is warranted.

Key words: developmental effects, infants, middle ear, wideband absorbance

Abbreviations:

ABR – Auditory Brainstem Response

DPOAE- Distortion product otoacoustic emissions

HFT – High frequency tympanometry

OAE – Otoacoustic emissions

SD – Standard deviation

WAI – Wideband acoustic immittance

WBA – Wideband absorbance

WBR – Wideband reflectance

Key words: developmental effects, infants, middle ear, wideband absorbance

6.3 Introduction

Assessing conductive disorders in young infants (aged 0 to 6 months) is a real challenge (Kei and Zhao, 2012) as there are, currently, no effective tools for detecting these disorders for this population. Nevertheless, wideband acoustic immittance (WAI) (Feeney et al., 2013), a physiological measure that provides information about the conduction properties of the outer and middle ear across a wide frequency range has been found to be a promising tool for detecting disorders in the conductive pathway (Feeney and Sanford, 2012).

Studies of WAI have often measured either wideband reflectance (WBR) or wideband absorbance (WBA) in humans. WBR is defined as the ratio of reflected power to incident power (Voss and Allen, 1994). To date, the vast majority of studies have focused on measuring WBR in young infants. In the present paper, the findings of these studies are discussed in terms of WBA (1-WBR) because the pattern of absorbance results is similar to the traditional admittance pattern which is familiar to

clinicians. WBA ranges from 1 (where all of the sound energy is absorbed by the middle ear) to 0 (where all the energy is reflected back from the middle ear) (Stinson, 1990).

The WBA test measures the absorbance at either ambient or variable pressure using a wideband stimulus, such as a click or chirp. WBA has been found useful in the evaluation of middle ear function in neonates (Keefe et al, 2000; Sanford et al, 2009; Hunter et al, 2010; Aithal et al, 2013), infants and young children (Jeng et al, 1999; Margolis et al, 2000; Hunter et al, 2008), and older children and adults (Piskorski et al, 1999; Feeney et al, 2003; Keefe & Simmons, 2003; Allen et al, 2005).

Studies of WBA in infants have found changes to WBA with age (e.g., Keefe et al, 1993; Sanford and Feeney, 2008). These changes may be attributed mainly to developmental effects of the peripheral auditory system. In particular, the acoustical properties of the outer and middle ear undergo significant changes during this fast developing period of the auditory system. The developmental aspects of the outer and middle ear, as summarized by Wilson (2012), include: (1) changes in the shape and increase in length and diameter of the external auditory canal (Saunders et al, 1983; Keefe et al, 1993; Qi et al, 2006), (2) decrease in the cartilaginous portion and increase in the bony portion of the canal wall (McLennan and Webb, 1957; Fung, 1993), (3) changes in tympanic membrane orientation (Eby and Nadol, 1986; Ikui et al, 1997; Qi et al, 2006), (4) reduction in the thickness of the tympanic membrane (Ruah et al, 1991), (5) reduction in vascular and cellular content (Richany et al, 1954), (6) increase in the volume of the tympanic cavity (Ikui et al, 2000), (7) increase in pneumatisation and growth of the temporal bone as a whole, particularly the mastoid process (Anson et al, 1955), and (8) increase in weight and size of the ossicles (Anson and Donaldson, 1981).

As developmental changes in early infancy influence the acoustical properties of the ear, it is very important to have accurate, age appropriate WBA measures in order to determine the outer and middle ear status in infants. This would be useful during newborn hearing screening, rescreening and follow up, as well as assisting in accurate interpretation of otoacoustic emission (OAE) and auditory brainstem response (ABR) results (Sanford and Feeney, 2008).

Normative WBA data for neonates have recently been established (Keefe et al, 2000; Shahnaz, 2008; Sanford et al, 2009; Hunter et al, 2010; Merchant et al, 2010; Aithal et al, 2013). In comparison, WBA data sets for young infants (1-12 months) are limited (Feeney and Sanford, 2012). Several studies have shown that, at all ages, WBA is the lowest at frequencies below 1 kHz and above 4 kHz and highest in the frequency region between 1 and 4 kHz, which corresponds to the most effective frequency region of the acoustic transfer function (Keefe et al, 1993; Hunter et al, 2008; Sanford and Feeney, 2008; Sanford et al, 2009). Nevertheless, the results of previous studies have been equivocal regarding the changes in WBA with increasing age especially in the first 12 months of life (Kei et al, 2013).

Keefe et al (1993) were the first to compare WBA among adults, infants aged 1-, 3-, 6-, 12- and 24 months. For all infant age groups, WBA was lowest at low frequencies and highest between 1 and 4 kHz. There was a significant age effect below 1 kHz with decreasing WBA up to six months of age. Keefe et al suggested that the development of the infant's external and middle ear (such as growth of the area and length of the ear canal), resonance in the ear canal walls of younger infants and growth of middle ear cavities were the contributing factors for the changes in WBA with age. Differences in WBA between 24-month-old infants and adults suggested that maturation of the peripheral auditory system is not complete by 24 months of age. Although healthy infants with no history of outer or middle ear disorders were included in Keefe et al's study, there were no other tests performed on the infants to check for conductive conditions in the outer and middle ear prior to their inclusion.

Sanford and Feeney (2008) provided useful information on the maturation effects of the peripheral auditory system on WBA. They measured WBA in 60 healthy, full-term infants with 20 infants in each age group of 4-, 12-, and 24- weeks with normal conductive pathway as determined by a pass in distortion product otoacoustic emissions (DPOAE) and high frequency (1-kHz) tympanometry (HFT). They found a 30% decrease in the mean WBA for frequencies from 0.25 to 0.75 kHz with increasing age, which concurred with the findings of Keefe et al (1993). Likewise, Werner et al (2010) analysed WBA in 198 infants aged 2 to 3 months, 260 infants aged 5 to 9 months and 210 healthy adults with normal middle ear function as determined by a pass in traditional 226-Hz tympanometry. They reported a significant

age effect for WBA from 0.25 to 3 kHz with WBA decreasing progressively with age. However, 226-Hz tympanometry has been found to be unreliable in assessing middle ear function in infants up to six months of age (Paradise et al, 1976; Holte et al, 1990; Hunter and Margolis, 1992; Williams et al, 1995; McKinley et al, 1997).

In contrast, Merchant et al (2010) investigated WBA in seven neonates (12 ears) and eleven 1-month-old infants (19 ears). Except for a slight difference at 2000 Hz between newborns and 1-month-old infants, there was no significant distinction in WBA and transmittance between the two age groups across frequencies. Similarly, Hunter et al (2008) studied 97 infants and children, aged between 3 days and 47 months, with 138 ears classified as normal hearing status and 21 ears having 'poor ear' status as determined by a combination of DPOAE, tympanometry (226- or 1000-Hz) along with pneumatic otoscopy performed by a physician. The infants were pooled into five age ranges, namely, 3 days to 2 months, 3 to 5 months, 6 to 11 months, 12 to 23 months and 24 to 47 months. Hunter et al did not find any significant difference in WBA as a function of age except at 6 kHz. Hunter et al. suggested that differences in probe design and calibration methods might account for differences in the developmental trends with age.

In view of the disagreement regarding developmental trends in WBA, Kei et al (2013) suggested that clinicians should be cognizant of variations in WBA measurements caused by developmental changes in anatomy and physiology, and recommended further research to replicate the findings of previous studies with appropriate reference standards for normal middle ear status. If the WBA measure is to be considered as a diagnostic tool for evaluating the function of the conductive pathway in young infants, it is imperative that age appropriate norms be developed. Age appropriate norms will aid in understanding the developmental trends of WBA and will be useful during evaluation of test performance of WBA in young infants. Additionally, due to significant developmental changes during early infancy, there is a need to include infants at various age intervals in order to more closely describe developmental effects of the outer and middle ear (Sanford and Feeney, 2008). The objective of the present study was to compare WBA in healthy newborns and infants aged 1-, 2-, 4- and 6- months.

6.4 Method

6.4.1 Subjects and test environment

Ethical approval for the study was obtained from the Townsville Health Service District Institutional Ethics Committee and the University of Queensland Behavioural and Social Sciences Ethical Review Committee. Parents of healthy neonates at the maternity ward of Townsville Hospital were informed of the study by nurses prior to the hearing screening of neonates. Parents consented for their babies to participate in the study by completing a consent form approved by the above Ethics committees. All infants were born at term, with normal birth weight and no medical complications. Testing was offered at birth with follow up at one, two, four and six months of age. Nevertheless, very few infants attended more than one follow up appointment. When an infant attended more than one follow up appointment, data obtained at only one of the appointments were included for analysis. Hence, this study describes cross-sectional data of infants at various age intervals. The number of subjects enrolled included 50 newborns, 36 infants at 1 month of age, 30 infants at 2 months of age, 33 infants at 4 months of age and 28 infants at 6 months of age. All infants enrolled in the study were Caucasian. None of the infants in the present study participated in the Aithal et al (2013) study.

All measurements with the neonates were performed in a quiet room in the maternity unit. Evaluations with the infants aged one to six months were performed in a sound treated room at the Audiology department. Mean ambient noise levels in the maternity room and sound booths were 35.7 and 32.0 dB A, respectively. Infants at all ages were seen after feeding while in natural sleep or in an awake but quiet state.

For infants in each age group, only the ears that passed both HFT and DPOAE screening tests were included in the study. Inclusion of HFT in the test battery is imperative to identify middle ear disorders that are not detectable by the otoacoustic emission test (Driscoll et al, 2001). Details of infants included in the study are provided in Table 6.1. The total number of infants included in the study is less than the total number recruited because some infants failed either one or both tests in the test battery or data were not available for all three tests (HFT, DPOAE or WBA test). With neonates, although both ears of most neonates passed the screening test battery, only one ear per infant was randomly chosen for the study.

Table 6.1: Details of infants included in the study (inclusion criteria – pass in HFT and DPOAE)

Age Group (in months)	Number of subjects			Number of ears			Age		
	Male	Female	Total	Right	Left	Total	Mean	SD	Range
0	14	21	35	18	17	35	41.49 hours	14.82	21.51-70.37 hours
1	10	6	16	15	14	29	32.81 days	5.82	29-47 days
2	11	5	16	14	15	29	58.31 days	3.55	54-66 days
4	11	4	15	15	13	28	124.13 days	8.53	105-135 days
6	11	3	14	14	13	27	179.43 days	5.00	169-189 days

6.4.2 Procedure

6.4.2.1 Screening test battery

A test battery consisting of HFT and DPOAE tests was used to determine the status of the outer and middle ear. While a pass in the test battery provides some assurance of an unobstructed conductive pathway, it should not be regarded as a gold standard for detecting ears with a conductive condition in view of the limitations of the test battery when used with young infants. As the pass criteria of the individual tests for all age groups were not available, the pass criteria for use with neonates were adopted in the present study.

6.4.2.1.1 Tympanometry.

HFT was performed using a GN Otometrics Otoflex acoustic immittance device with a 1000 Hz probe tone. During the test, the admittance (Y) was measured as the pressure was changed from +200 to -400 daPa at a rate of 400 daPa/sec. A visual classification system was used to classify the tympanometric results. The pass criterion was a single positively peaked tympanogram as described by Baldwin (2006).

6.4.2.1.2 DPOAE.

DPOAE was performed using a Biologic Navigator Plus. DPOAEs were measured in response to pairs of primary tones with F2 set at 2, 3, 4, and 6 kHz. The F2/F1 ratio was 1.2 for each primary pair. The stimulus level of F1 was 65 dB SPL and F2 was 55 dB SPL. Pass criteria included (i) a noise level of less than 0 dB SPL, (ii) the difference between the amplitude of the emission and associated noise floor to be at least 6 dB in at least three out of four frequencies from 2 to 6 kHz (Sanford et al, 2009; Hunter et al, 2010), and (iii) a DPOAE magnitude with a level of at least -6 dB at 2, 3, 4 and 6 kHz (Sanford et al, 2009; Merchant et al, 2010).

6.4.2.1.3 WBA.

WBA was measured using a prototype research system developed by Interacoustics A/S (Denmark). The Reflwin computerised system consisted of a Windows-based computer, a 24 bit resolution sound card, a pressure pump and controller system contained in an acoustic immittance instrument (AT235), and custom software for stimulus generation and data acquisition. Calibration was performed every day (Keefe and Simmons, 2003; Sanford and Feeney, 2008) at ambient pressure to determine the source reflectance and incident sound pressure associated with the probe and its transducers based on acoustic measurements in four rigid-walled, cylindrical calibration tubes that were open at one end and closed at the other end with a steel rod. The infant calibration tubes had lengths of 232.11 and 5.319 mm, each with a diameter of 4.8 mm. The adult calibration tubes had lengths of 290.50 and 8.10 mm, each with a diameter of 7.9 mm. Root mean squared (RMS) reflectance error function was generated to determine the error in the acoustical estimate of the length of each tube relative to the acoustic wave propagation model. An RMS reflectance error of less than 0.009 was required for a successful calibration. Any calibration that did not meet the criteria was repeated following probe reinsertion.

Ambient WBA measurements were obtained by recording acoustic response to clicks, presented at 55 dB SPL and at a rate of one click per 46 msec. Responses from a total of 16 clicks were averaged for each measurement and reflectance was calculated for each response. The WBA response consisted of 16 data points (at 1/3 octave frequencies from 0.25 to 8 kHz). A visual display with high absorbance at

frequencies below 1 kHz served as an on-screen prompt that alerted the tester to a potential probe leak. A visual prompt also alerted the tester if the noise level was high. The data acquisition was very quick and the typical test time in a sleeping or well settled infant was less than 10 seconds.

6.5 Results

6.5.1 WBA within age groups

A mixed model analysis of variance (ANOVA) was fitted to the WBA data obtained from each age group with gender and ear as between-group factors and frequency as a within-group factor. An alpha level of 0.05 was used for all analyses. The Greenhouse and Geisser (G-G) (1959) approach was used to compensate for the violation of compound symmetry and sphericity.

As illustrated in Table 6.2, ANOVA results showed a significant main effect for frequency for each age group. The effects of ear and gender and their interactions were not significant for any age group. WBA data from right and left ears were treated as independent samples.

Table 6.2: Analysis of variance results illustrating significant effect of frequency across all age groups

Age	Type III sum of square	Df	Mean square	F	Significance	Observed Power
0-months	6.31	4	1.49	25.18	0.00	1.00
1-month	4.33	3	1.84	18.67	0.00	1.00
2-month	3.15	3	0.99	6.31	0.00	0.96
4-month	8.89	3	3.27	27.15	0.00	1.00
6-month	7.84	3	2.47	21.26	0.00	1.00

6.5.2 WBA across age groups

Figure 6.1 illustrates the pattern of median WBA results for 0-, 1-, 2-, 4- and 6- month-old infants at 16 one-third-octave frequencies. WBA results for the 0-, 1-, and 2-month-old infants exhibited a multi-peaked pattern, with the peaks occurring around 1.5 and 5 kHz. This multi-peaked pattern was less distinct for the 4- and 6-month-old infants and shifted to a single, broad-peaked pattern with the peak occurring between 2 and 5 kHz.

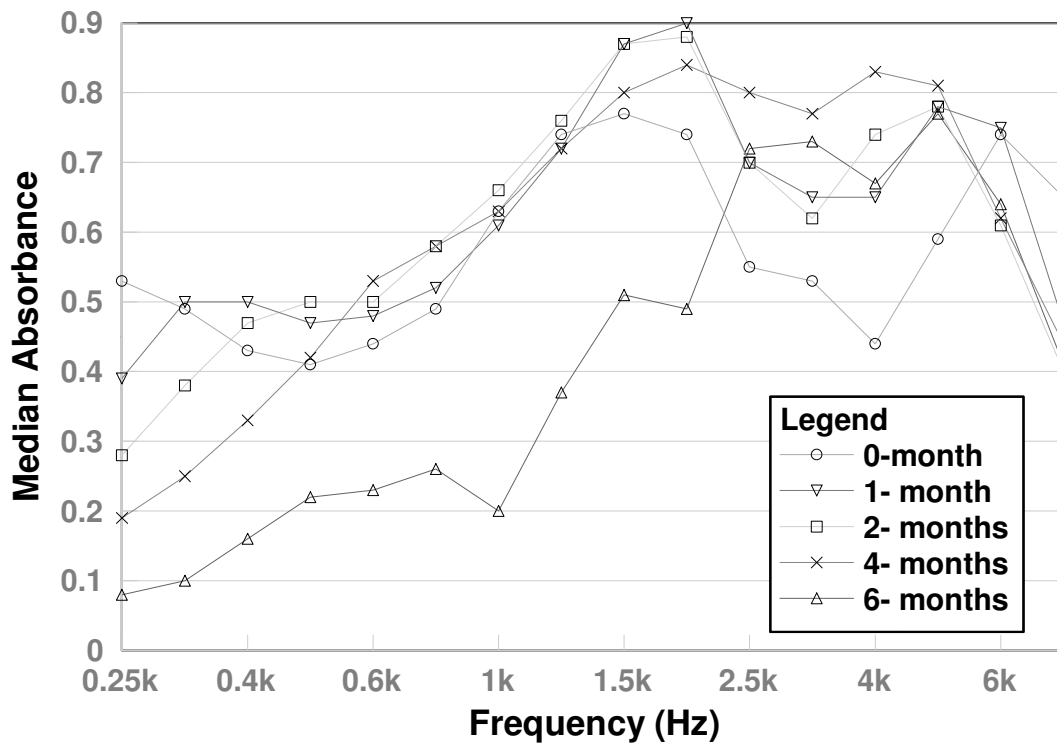


Figure 6.1: Median WBA in newborns (0-month-old), 1-, 2-, 4- and 6-month-old infants.

At all ages, WBA was highest between 1 and 5 kHz, and lowest between 0.25 and 0.8 kHz and between 6 and 8 kHz. The median WBA of 6-month-old infants was lowest especially between 0.25 and 1.5 kHz compared to infants in 0-, 1-, 2- and 4- month age groups. The median WBA between 2 and 4 kHz was highest in infants aged 4- months, followed by infants aged 1-, 2- and 6- months and lowest in 0-month-old infants. On the other hand, the median WBA between 5 and 8 kHz decreased with increasing age with the median WBA of 6-month-old infants being the lowest and that of 0-month-old infants being the highest in this frequency range.

Figure 6.2 illustrates the standard deviation (SD) of WBA across frequencies for the age groups. SDs were calculated as a measure of variability since the mean and median values of WBA were similar for all age groups. The SDs generally increased with increase in frequency for 0-, 1-, 2-, and 4- month old infants. The SDs for 0- and 1- month old infants were lower than that of 2- and 4-month-old infants. On the other hand, with the 6-month-old infants, the SD increased from 0.25 to 1 kHz and remained the same between 1 and 2 kHz and decreased from 2 to 3 kHz and again

increased from 4 to 8 kHz. The SDs for 6-month-old infants were similar to those for 0- and 1-month-old infants at frequencies between 0.25 and 0.6 kHz and between 2.5 and 8 kHz. The SDs for 6-month-old infants between 1.25 and 2 kHz were higher than those of the other age groups.

To compare WBA among the five age groups, a mixed model ANOVA was performed with age as between-subject factor and frequency (16 levels) as a within-subject factor. An alpha level of 0.05 was used for analysis. There were significant main effects for age [$F(4, 143) = 15.01, p=0.00$] and frequency [$F(5, 766) = 132.77, p=0.00$] and age x frequency interaction [$F(21, 766) = 9.75, p= 0.00$]. This means that the pattern of WBA was significantly different across the age groups. *Post hoc* analysis using Bonferroni correction was performed at each of the 16 one-third-octave frequencies to examine the effect of age on WBA. Table 6.3 shows the results of the Post hoc analysis. A developmental trend is evident with both the 0- and 6-month-old infants being significantly different to other age groups at most of the frequencies. In general, the WBA of 0-month-old infants was significantly different to that of 1-month-old infants between 1.5 and 5 kHz. The WBA of 0-month-olds was significantly different to that of 2- and 4-month-old infants at all frequencies except between 1 and 1.5 kHz. The WBA of 1-month-old infants was significantly different to that of 2-month-old infants only at 0.8 kHz. The WBA of 4-month-old infants was significantly different from that of 1- and 2-month-old infants mainly in the low frequencies from 0.25 to 0.4 kHz but similar at other frequencies. The WBA of 6-month-old infants was significantly different to the WBA of 0-, 1-, 2- and 4- month-old infants at most frequencies.

6.5.3 Comparison of WBA across different studies

Since there are very few studies on age specific WBA norms for infants, it is important to compare the results between available studies to identify similarities and differences. WBA results for neonates from Hunter et al. (2010) (mean age 29 hours, SD 15.5 hours), Merchant et al (2010) (age 3 to 5 days) and Aithal et al (2013) (mean age 46 hours, SD = 21 hours) are plotted along with median WBA data from the present study in Figure 6.3. The results from the Hunter et al (2010) and Aithal et al (2013) studies are consistent with the results of the present study. Results from the

Merchant et al (2010) study were similar to findings of other studies up to 2 kHz with a large disparity observed between 2.5 and 8 kHz.

Table 6.3: Results of a Post hoc analysis using multiple comparisons with Bonferroni correction comparing WBA between age groups

Frequency (kHz)	Groups by Age (months)									
	0-1	0-2	0-4	0-6	1-2	1-4	1-6	2-4	2-6	4-6
0.25	0.00*	0.00*	0.00*	0.00*	0.44	0.00*	0.00*	0.00*	0.00*	0.00*
0.3	0.38	0.04*	0.00*	0.00*	0.22	0.00*	0.00*	0.00*	0.00*	0.00*
0.4	0.06	0.31	0.00*	0.00*	0.74	0.00*	0.00*	0.00*	0.00*	0.00*
0.5	0.16	0.00*	0.66	0.00*	0.07	0.61	0.00*	0.08	0.00*	0.00*
0.6	0.45	0.37	0.01*	0.00*	0.72	0.07	0.00*	0.27	0.00*	0.00*
0.8	0.95	0.01*	0.39	0.00*	0.01*	0.36	0.00*	0.19	0.00*	0.00*
1	0.69	0.45	0.40	0.00*	0.27	0.60	0.00*	0.18	0.00*	0.00*
1.25	0.77	0.80	0.31	0.00*	0.99	0.22	0.00*	0.28	0.00*	0.00*
1.5	0.01*	0.16	0.82	0.00*	0.44	0.06	0.00*	0.32	0.00*	0.00*
2	0.00*	0.04*	0.02*	0.89	0.14	0.14	0.00*	0.92	0.053	0.03*
2.5	0.00*	0.04*	0.00*	0.00*	0.35	0.29	0.96	0.11	0.40	0.30
3	0.00*	0.06	0.00*	0.00*	0.60	0.04*	0.68	0.04*	0.43	0.10
4	0.00*	0.00*	0.00*	0.00*	0.47	0.01*	0.06	0.15	0.47	0.36
5	0.01*	0.19	0.04*	0.83	0.28	0.72	0.03*	0.53	0.35	0.12
6	0.85	0.09	0.01*	0.00*	0.10	0.02*	0.00*	0.50	0.00*	0.05*
8	0.13	0.00*	0.00*	0.00*	0.13	0.10	0.00*	0.92	0.00*	0.00*

*Significant at 0.05 level

Median WBA results for 1-month-old infants from the present study are plotted along with results from Keefe et al (1993) (age 1 month), Sanford and Feeney (2008) (mean age = 4.5 weeks, SD = 0.34) and Merchant et al (2010) (age 28 to 34 days) in Figure 6.4. Apart from some differences in the magnitude of WBA obtained across studies, the WBA pattern was consistent across all the four studies. Figure 6.5 illustrates the median WBA results for 2-month-old infants from the present study along with the results from Prieve et al (2013) for 10-week-old infants. Despite the large magnitude of WBA in the present study, the WBA pattern was again similar across both the studies.

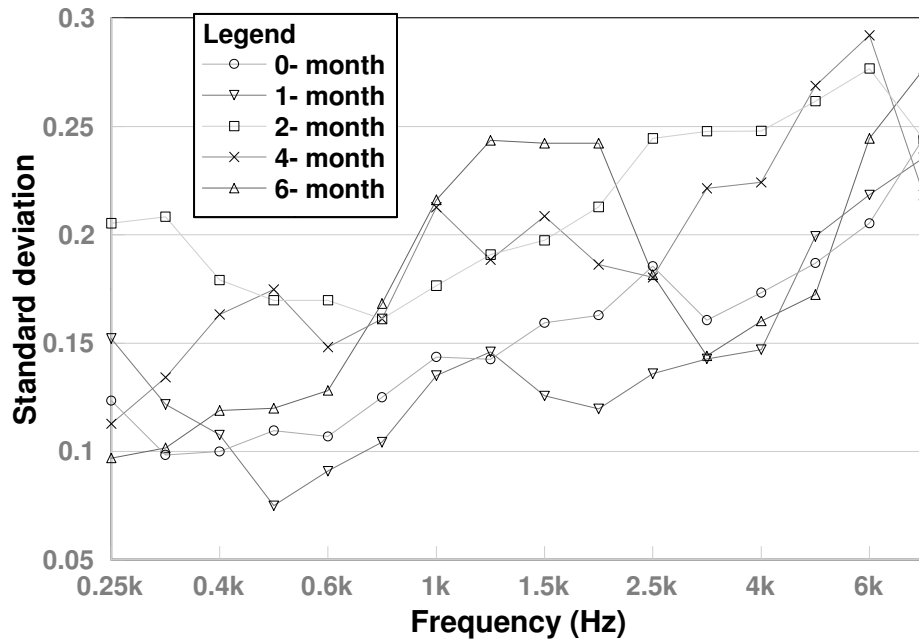


Figure 6.2: Standard deviations of WBA for frequencies from 0.25 to 8 kHz in newborns (0-month-old), 1-, 2-, 4- and 6-month-old infants

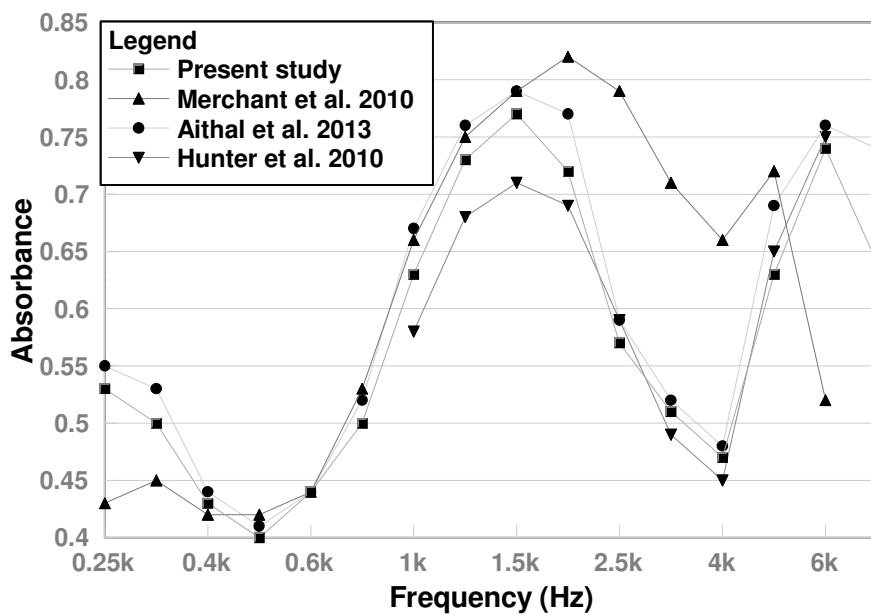


Figure 6.3: Comparison of WBA in neonates across four studies

Median WBA results for 6-month-old infants from the present study are plotted along with results from Keefe et al (1993)(age 6 months) and Sanford and Feeney (2008) (mean age 24.1 weeks, SD =0.31 weeks) in Figure 6.6. Although the general pattern of WBA obtained was the same across the frequencies, there were

differences in the magnitudes of WBA especially from 0.5 to 2 kHz. Across the entire frequency range, the WBA values were highest in Sanford and Feeney's (2008) study and lowest in the present study.

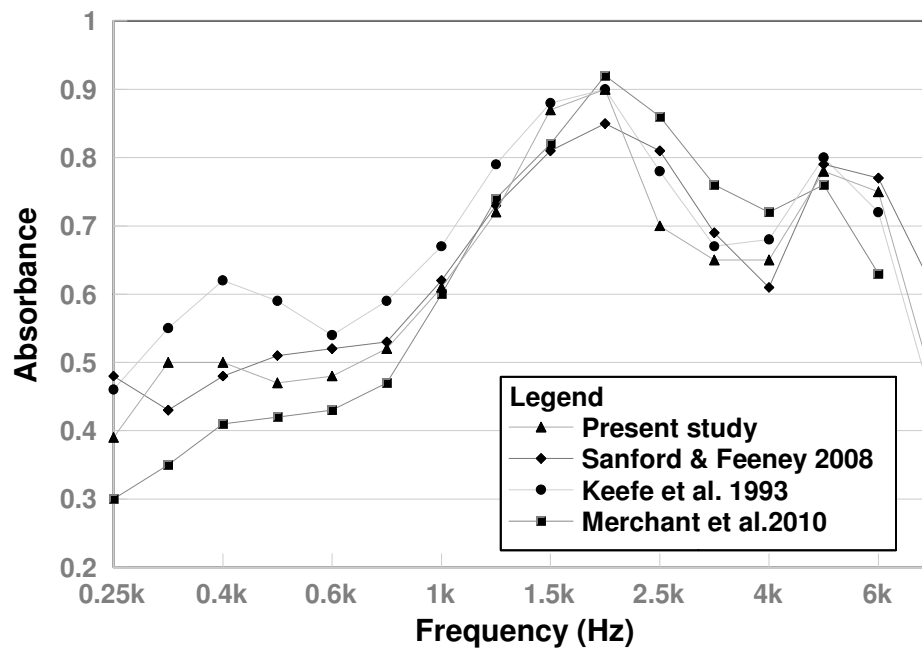


Figure 6.4: Comparison of WBA in 1-month-old infants across four studies

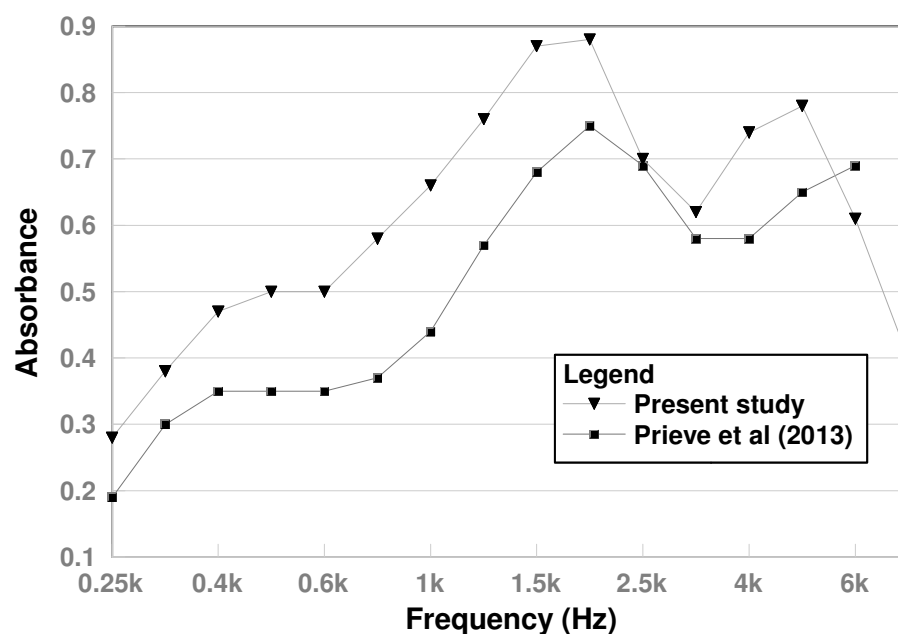


Figure 6.5: Comparison of WBA in 2-month-old infants between Prieve et al. (2013) and the present study

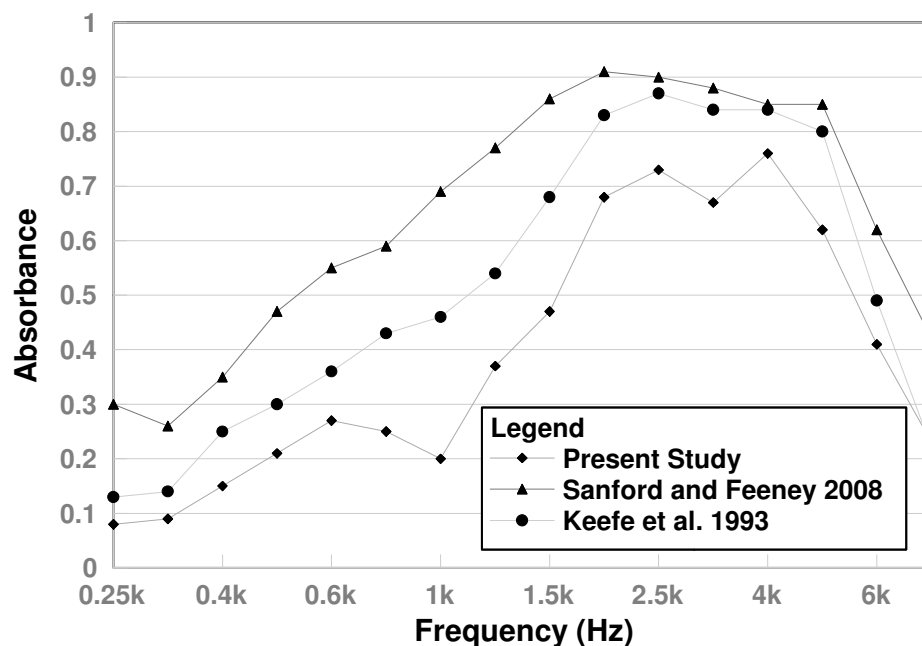


Figure 6.6: Comparison of WBA in 6-month-old infants across three studies

6.6 Discussion

The objective of the present study was to compare WBA obtained from young infants with a view to examine the developmental characteristics of infants in their first six months of life. Normative data were provided for each age group (Appendix A). These data can be used as a reference for detecting disorders in the sound conduction pathway (outer and middle ears) in young infants.

The results revealed significant changes in WBA with age, indicating maturation effects. First, the multi-peaked pattern of absorbance seen at 0, 1 and 2 months of age became less prominent with a shift towards a single peak at 4 and 6 months of age (Figure 1). Similar results have been reported by Sanford and Feeney (2008) who found that the multi-peaked effect across frequency at 4 weeks of age became less distinct for the 12- and 24-week-old infants and shifted towards a single peaked function.

Second, the median WBA decreased with increasing age, especially for frequencies from 0.25 to 0.5 kHz (Figure 1). The median WBA of 1-month-old infants was the highest, while the WBA of 6-month-old infants was the lowest. The differences in median WBA were the smallest between newborns and 1-month-old infants, and between 2- and 4-month-old infants, but greatest between 1-month-old and 6-month-old infants. These results are similar to the findings of Keefe et al (1993) who reported higher WBA in 1-month-old infants than older infants aged 3 and 6 months. Factors contributing to developmental differences include growth in the outer and middle ear, and stiffening of the compliant ear-canal wall which is more resistant to changes in pressure with age (Keefe et al, 1993; Sanford and Feeney, 2008).

Third, the newborns had lower WBA than other infants (aged 1-6 months) in the high frequencies between 2.5 and 5 kHz (Figure 1). This is a distinctive feature of the developmental aspects of the newborns. Acoustic effects at high frequencies in infancy have been attributed to changes in the properties of the ossicular chain (Saunders et al, 1983). Eby and Nadol (1986) reported that while the neonatal ossicles have obtained adult dimensions, they are histologically immature and composed of cartilage. Additionally, temporal bone studies suggest that residual mesenchyme adhering to the ossicular chain can lead to mass loading of the ossicles. From one month of age onwards, infants showed an increase in WBA in the high frequencies to reach a level at par with that of the older infants. This phenomenon may be explained in terms of the diminishing mass and resistance of the middle ear in older infants due to changes in bone density of the ossicles and loss of mesenchyme and fluids in the middle ear (Olszewski, 1990; Richany et al, 1954).

Finally, the 6-month-old infants had significantly lower WBA than other age groups in the low to mid frequencies (0.25 to 2 kHz) (Figure 1). Similarly, Sanford and Feeney (2008) and Keefe et al (1993) have reported the most dramatic decrease in WBA in low frequencies for infants between 4 and 12 weeks of age. The low WBA of the 6-month-old infants in this frequency range indicates increased stiffness of the outer and middle ear. The increase in stiffness may be attributed to a myriad of factors in the maturation process including the ossification of the inner two-thirds of the ear canal, changes in the orientation and fibre structure of the tympanic membrane, fusion of the tympanic ring, and tightening of the ossicular joints.

There was no significant difference in WBA between the right and left ears for any age group in the present study. In contrast, other studies have reported small, albeit significant, differences in WAI between right and left ears. For instance, in their study of eight neonates and eleven 1-month-old infants, Merchant et al (2010) found a significant difference in WBA between right and left ears. Similarly, Werner et al (2010) studied 458 infants from two to nine months of age and found that right ears had lower WBA than left ears. In contrast, Keefe et al (2000) found that WBA was lower in left ears than in right ears for frequencies below 1.4 kHz, but higher in left ears at higher frequencies.

There were no significant differences in WBA values between males and females in any of the age groups in the present study. This result is in agreement with the findings of Werner et al (2010) and Merchant et al (2010) who found no gender effect in their studies. In contrast, Keefe et al (2000) found that WBA was lower for males than for females at frequencies below 2 kHz.

The present study found a significant frequency effect in WBA across all age groups with WBA being the highest in the frequency region between 1 and 4 kHz. These results are consistent with the findings of Keefe et al (1993), Vander Werff et al (2007), Sanford and Feeney (2008), Sanford et al (2009), Hunter et al (2010), Merchant et al (2010) and Werner et al (2010). The median absorbance obtained for various age groups in the present study compares favourably with those reported in previous studies (Figures 3-5). Although the WBA against frequency patterns were the same, there were differences in WBA across the studies. The discrepancies across studies may be attributed to differences in subject sampling, equipment, test conditions and reference standards for middle ear function.

6.6.1 Limitations of the study

Several limitations have been identified in the present study which may have affected the interpretation of results. First, the difficulty of completing all tests increased with the age of infants. Older infants were more likely to be tested while they were awake, and therefore, jaw movements and suckling might have contributed to variations in estimating WBA. Second, the sample size was small for neonates and

infants aged between 1 and 6 months. This limits the generalisation of results to the infant population. It is, therefore, necessary to develop age specific normative WBA data using large sample sizes and established middle ear reference standards such as diagnostic otoacoustic emissions. . Third, the use of test battery necessitated change of probes for each test which could disturb an infant. Further research is recommended using equipment that allows all the tests to be done using a single probe. Last, the use of a cross-section design of the study has restricted, to some extent, the investigation of maturation of the outer and middle ear of infants especially during the fast developing period. It was not possible to track developmental changes in an individual from birth to six months. Future studies may employ a longitudinal design to track developmental changes in WBA.

6.6.2 Conclusion

The present study provided evidence of maturation in the outer and middle ear of infants in their first 6 months of life. The WBA in the low to mid frequencies decreased with increasing age from birth to 6 months, indicating increasing stiffness due to growth in the outer and middle ear, and stiffening of the compliant ear-canal wall. From birth to 1 month, neonates showed significant increase in WBA in the high frequencies (≥ 2.5 kHz). The WBA continued to increase with age up to 4-6 months of age. These developmental changes may be attributed to changes in bone density of the ossicles and loss of mesenchyme and fluids in the middle ear, resulting in a smaller mass and resistive component of the middle ear. In particular, the WBA of 6-month-old infants was significantly different to that of 0-, 1-, 2- and 4- month-old infants at most frequencies, suggesting rapid development of the outer and middle ear during this period. Due to significant developmental changes in the first six months of life, it is necessary to develop age specific WBA normative data using a large sample for each age group.

6.7 References

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Chapter Seven: General Discussion and Conclusions

7.1 Introduction

WBA is reported to predict the outcomes of DPOAE better than HFT and, hence, recommended as a clinical tool during NHS. Nevertheless, clinical application of WBA requires development of normative data and evaluation of pathologic effects against robust reference standards of middle ear function as well as exploration of developmental effects and application with special populations where prevalence of middle ear dysfunction is high.

This chapter revisits the rationale and aims of the study (as described in chapter one) and discusses the main findings presented in the previous chapters (chapters two to six). Clinical application, limitations of the study, implications for further research in the area of WBA in neonates and young infants are also presented in this chapter.

7.2 Rationale For The Study (Revisited)

The current study was devised to study applications of WBA in the Australian context. Although NHS programs have been introduced in several states and territories in Australia for more than a decade, there are no published studies on the prevalence of conductive hearing loss and false positive rates in this population. In particular, it is important to determine the prevalence of conductive hearing loss in the Australian Aboriginal infants because this group is prone to otitis media. The study, described in Chapter 2, determined the prevalence of middle ear dysfunction and conductive hearing loss in Aboriginal and non-Aboriginal infants referred through a NHS program in Australia.

Due to its ability to assess the middle ear independent of the inner ear, WBA is suggested as a feasible tool to be used in conjunction with the NHS program (Keefe et al., 2003a, b; Hunter et al., 2010; Sanford et al., 2009; Vander Werff et al., 2009) or during diagnostic assessment of infants (Prieve et al., 2013). For successful application of WBA, development of norms is crucial for clinicians to distinguish normal ears from ears with a conductive condition. To date, normative WBA data

have been developed using DPOAE as a reference standard for middle ear function (Hunter et al., 2010; Merchant et al., 2010; Sanford et al., 2009). Nevertheless, DPOAE may not identify minor or sub-clinical middle ear pathologies (Kemp, 2002) and, hence, may not be an ideal gold standard (Hunter et al., 2010; Sanford et al., 2009). Perhaps, a test battery reference standard may provide a better measure of middle ear function than a single test reference standard (Mazlan & Kei, 2012). To date, development of normative WBA data based on a test battery reference standard has not been investigated. The study, described in chapter 3, provided normative WBA data in healthy neonates who passed a battery of tests including AABR, HFT, ASR, TEOAE and DPOAE.

Evaluation of test performance of WBA is challenging. Even though air and bone conduction (AC and BC) tone burst ABR may be regarded as a surrogate gold standard for comparison with WBA results, this threshold ABR measure requires long testing time to complete and is not commonly performed during the hearing screening period. In view of this, most studies circumvent this issue by using DPOAE as the gold standard because it is commonly used as a screening tool in NHS programs (Sangster, 2011). As mentioned, DPOAE may not accurately identify minor or sub-clinical conductive pathologies (Kemp, 2002) and may not serve as an ideal reference standard. Use of DPOAE in this manner represents a significant shortcoming in the evaluation of WBA (Sangster, 2011). Since myringotomy is not ethical or feasible in normal neonates, an alternate would be to determine the test performance against a battery of tests of middle ear function. The study, described in chapter 4, evaluated the test performance of WBA against various reference standards to determine whether WBA can provide a more effective alternate to either single tests or test batteries for determining the outer and middle ear status in neonates.

Australian Aboriginal children have high rates of OM that starts early in life. Nonetheless, there is limited research regarding the status of the middle ear of Aboriginal infants at birth. Previous studies have used 226 Hz tympanometry, otoscopy and TEOAE to evaluate middle ear function in young Aboriginal infants. Unfortunately, 226 Hz tympanometry, otoscopy and TEOAE do not accurately identify OM or conductive conditions in infants below six months of age. To date, there have been no studies that have used WBA to evaluate outer and middle ear

function in Aboriginal neonates. Additionally, differences in WBA have been reported between ethnic groups lending support to the development of ethnic specific norms for WBA (Shahnaz & Bork, 2006). The study, described in chapter 5, compared the WBA between Aboriginal and Caucasian neonates with and without middle ear dysfunction.

The outer and middle ear of infants undergoes significant developmental changes in the first few months of life. Differences in size and structure of the outer and middle ears relative to age could have an effect on the transmission of sound through the middle ear (Shahnaz, 2010). Hence, age specific normative WBA data need to be established in the first few months in order to more accurately assess outer and middle ear dysfunction in this population. The study, presented in chapter 6, described the developmental aspects of WBA for healthy infants in their first six months of life.

7.3 Aims of the Thesis (Restated)

Given the above rationales for the study, the aims of this thesis were to

- (1) Evaluate the prevalence of conductive hearing loss and middle ear pathology in infants referred by a NHS program in Australia and compare the prevalence rates of conductive hearing loss and middle ear pathology in Aboriginal and non-Aboriginal infants (see chapter 2).
- (2) Obtain normative WBA data in healthy neonates who pass a combination of tests including AABR, HFT, ASR, TEOAE and DPOAE (see chapter 3).
- (3) Compare test performance of WBA using individual measures such as AABR, HFT, TEOAE or DPOAE and a combination of tests (Eg: HFT+DPOAE) to determine whether WBA can provide a more effective alternate to either single tests or test battery (composite) reference standards for determining the outer and middle ear status in neonates (see chapter 4).
- (4) Compare WBA measures obtained from healthy Aboriginal neonates with that obtained from non-Aboriginal neonates (see chapter 5).
- (5) Conduct a cross sectional study on a sample of normal infants to determine the developmental trend of WBA results at birth and at 1, 2, 4, and 6 months of age (see chapter 6).

7.4 Hypotheses of the Study (Restated)

The present investigation of WBA and middle ear status in young infants contained four null hypotheses to be tested. They were:

H₀1: There will be no significant difference in the prevalence of conductive hearing loss between Aboriginal and Caucasian infants who are referred for diagnostic evaluation through a NHS program.

H₀2: There will be no significant difference in the test performance of WBA between single tests and test battery reference standards.

H₀3: There will be no significant difference in WBA results between Aboriginal and Caucasian neonates.

H₀4: There will be no significant age effects on WBA results obtained from infants aged from 0- to six months.

7.5 Discussion of the Main Findings

7.5.1 Middle ear pathology and conductive hearing loss in neonates

In spite of high rates of middle ear dysfunction in Australian Aboriginal children, at the commencement of this study, there were no data available on the prevalence of middle ear pathology and conductive hearing in infants referred through a NHS program in Australia. Therefore, this study was undertaken to determine and compare the prevalence of conductive hearing loss and middle ear pathology between Australian Aboriginal and Torres Strait Islanders (ATSI) and non-ATSI infants referred through an Australian NHS program.

Retrospective chart review of 234 infants referred to the Audiology department of the Townsville Hospital, a tertiary referral centre for north Queensland, was conducted. The mean age at the time of first diagnostic assessment was 47.5 days (SD = 31.30, range = 2 – 121 days). A battery of tests including HFT, TEOAE and ABR was performed to determine middle ear pathology with normal hearing or with conductive hearing loss. Prevalence of conductive hearing loss was found to be 2.97 per 1000 which was 1.8 times that of sensorineural hearing loss (1.64 per thousand). This was in agreement with findings of Boone et al. (2005) who also found prevalence of conductive hearing loss to be 1.8 times that of sensorineural hearing loss in infants

referred through an American NHS program. There were differences in the prevalence rate of conductive hearing loss between the present study and those reported in the literature, and the differences were attributed to differences in the screening methods used in the hearing screening programs.

ATSI infants had a higher prevalence of middle ear pathology with 44.45% (24 of 54) of ATSI and 28.66% (45 of 171) of non-ATSI infants demonstrating middle ear pathology with normal hearing or with conductive or mixed hearing loss. The prevalence of conductive hearing loss was twice as high in ATSI infants (19 of 54, 35.19%) compared to non-ATSI infants (28 of 157, 17.83%). Additionally, ATSI infants showed poor resolution of conductive hearing loss with 75% (12 of 16) of ATSI and 27.78% (non-ATSI) infants demonstrating persistent conductive hearing loss. Consequently, the null hypothesis (H_0) that predicted no significant difference in the prevalence of conductive hearing loss between Aboriginal and Caucasian infants referred for diagnostic evaluation through a NHS program was confidently rejected.

The findings of this study supported the use of an adjunct tool such as WBA to determine the status of the middle ear at the time of screening to facilitate prioritisation of infants for evaluation and streamline the management strategies for the respective types of hearing loss. The study also recommended longitudinal studies to evaluate natural history and resolution of MEE in the first twelve months of life to determine whether MEE is a precursor for later persistent middle ear infections.

7.5.2 Normative WBA measures in neonates

As mentioned earlier and detailed in Chapter Three, DPOAE is often used to develop normative WBA data. Since DPOAE is not an optimal tool of middle ear function, the aim of the second study in the thesis was to develop normative WBA measures in healthy neonates using a stringent reference standard to identify conductive conditions. WBA at ambient pressure was measured between 250 and 8000 Hz in one third octave frequencies in 66 infants (mean age = 46.0 hours, SD = 21.0, range = 13.3 – 116.5 hours) who passed a test battery of AABR, HFT, ASR, TEOAE and DPOAE.

The results showed no significant ear or gender difference in WBA although a significant difference in WBA among frequencies was observed. Various percentile values (0, 5, 10, 25, 50, 75, 90, 95, 100) were developed. The median absorbance reached a maximum of 0.76 to 0.79 between 1000 and 2000 Hz but decreased from 0.41 to 0.55 below 1000 Hz and 0.48 to 0.76 above 2000 Hz. The inter-quartile range (25th to 75th percentiles) at 2000 Hz and above was generally greater than that at frequencies below 2000 Hz. A general trend of increasing normative range (10th to 90th percentiles) with frequency was observed. Adjacent frequencies that did not differ significantly from each other were averaged to obtain absorbance area indices (AAIs). The AAIs determined in this study were 250 to 310, 400 to 800, 1000, 1250 to 2000, 2500, 3000 to 4000 and 5000 to 8000 Hz. The absorbance values obtained in this study were in agreement with studies by Shahnaz (2008) and Hunter et al. (2010), although the magnitude of absorbance in the 1000 to 4000 Hz region was smaller in the present study.

The requirement that all neonates should pass a test battery constituted a new robust reference standard for evaluating middle ear function in neonates without resorting to invasive procedures. Normative data developed in this study could be used as a reference to determine the outer and middle ear status of neonates.

7.5.3 Evaluation of test performance of WBA

Although the use of DPOAE as a reference standard for evaluating the test performance of WBA is reported to be a limitation (Sangster, 2011), to date, there are no studies that have compared the test performance of WBA with other reference standards of middle ear function. In the third study, test performance of WBA on 298 ears of 192 neonates was evaluated against nine reference standards that included four single tests (AABR, HFT, TEOAE and DPOAE) and five test batteries (HFT+TEOAE, HFT+DPOAE, TEOAE+DPOAE, HFT+TEOAE+DPOAE and AABR+TEOAE+DPOAE). Test performance of WBA was assessed in terms of its ability to identify conductive conditions in neonates across each reference standard.

The AROC of WBA for the test battery reference standards, were, in general, significantly higher than that for single tests. The test performance of WBA measured

against the HFT+TEOAE+DPOAE, HFT+TEOAE+DPOAE or HFT+DPOAE test battery reference standards was higher than that of single test batteries. In comparison, the AROC of WBA for HFT and AABR reference test standards were lower than AROC of WBA for other test batteries. Consequently, the null hypothesis (H_{02}) which predicted that there will be no significant difference in the test performance of WBA between single tests and test battery reference standards was rejected.

The region between 1000 and 2500 Hz provided the best discriminability to evaluate the conductive status compared to other frequencies. The median WBA for the pass group ranged between 0.40 and 0.76, with two maxima occurring at 1500 and 6000 Hz across all reference standards. The median WBA for the fail group showed a relatively flat pattern across the entire frequency range for all reference standards except HFT. The ears that passed each of the reference standards had significantly higher absorbance than the ears that failed.

The low performance of WBA against the single test reference standards suggests that a single test (such as AABR, HFT or OAE) per se may not accurately diagnose conductive condition in neonates. A plethora of reasons may account for the low performance. These may include: (1) the pass criteria of single tests were not optimal, (2) the tests are not sensitive to slight/mild conductive hearing losses, (3) some tests were susceptible to environmental and/or physiologic noise that could confound results, and (4) the tests provided limited clinical information about the properties of the conductive pathway. While acknowledging the limitations of single test reference standards, the use of a test battery consisting of HFT and OAE as a reference standard was recommended due to improved test performance compared to single test reference standards. WBA could provide a more effective alternate to a combination of tests for determining the outer and middle ear status in neonates. This finding is promising as it suggests that WBA is a valid measure of outer and middle ear function. Therefore, it has great potential to be used as an adjunct tool of middle ear function in newborn hearing screening programs or during diagnostic assessment.

7.5.4 WBA in Australian Aboriginal and Caucasian neonates

Although the prevalence of middle ear dysfunction is high in Australian children, there is very limited research on their middle ear status at birth. Although

conductive dysfunction at birth related to otitis prone conditions in the first year of life has been documented in Caucasian infants (Doyle et al., 2004; Pereira et al., 2010), there have been no such studies with Aboriginal infants. Hence there is a need to investigate the middle ear status of Aboriginal neonates using an efficient clinical tool such as WBA. A comparison of WBA results between Aboriginal and Caucasian neonates may reveal ethnic variances. The fourth study in the thesis explored the efficiency of the conductive pathway (outer and middle ear) using WBA between Aboriginal and Caucasian neonates at birth.

Testing was performed on 32 Aboriginal neonates and 158 Caucasian neonates who passed or failed a test battery comprising HFT and DPOAE. Interestingly, both groups showed identical pass rates of 61% with the test battery. However, both Aboriginal and Caucasian neonates who failed the test battery had significantly lower WBA than their counterparts who passed the test battery. The WBA of Aboriginal neonates that passed the test battery was significantly lower than that of their Caucasian counterparts at frequencies between 400 and 2000 Hz. WBA of Aboriginal neonates that failed the test battery was significantly lower at frequencies between 1500 and 3000 Hz compared to their Caucasian counterparts. Hence, the null hypothesis (H_{03}) that predicted that there will be no significant difference in the WBA results between the Aboriginal and Caucasian neonates was confidently rejected.

This study provided convincing evidence that although both groups had equal pass rates as determined by a test battery containing HFT and DPOAE, Aboriginal neonates had significantly lower WBA than their Caucasian counterparts. The low energy absorbance in Aboriginal neonates compared to Caucasian neonates suggests that these infants may have significant outer/middle ear disorders that were identified by WBA but not detected by the test battery. It is likely that a greater proportion of Aboriginal neonates would have failed the screening if a more sensitive test, such as WBA, was included in the test battery. Since WBA is a sensitive test of middle ear function, addition of WBA to the test battery could improve the outcomes of hearing screening in this population. While the two ethnic groups showed significant differences in WBA, the factors contributing to the differences remain undetermined and require further investigation.

7.5.5 WBA in young infants

The rapid developmental changes in the outer and middle ear system of infants in the first few months of life warrant investigation of age related changes in the acoustic properties of the conductive pathway. The fifth study in the thesis used a prospective, cross-sectional design to investigate the developmental effects of WBA in the first six months of life. WBA was measured in 35 ears of neonates, 29 ears of one-month-olds, 29 ears of two-month-olds, 28 ears of four-month-olds and 27 ears of six-month-olds who passed HFT and DPOAE. There were no significant ear effects or gender effects on WBA across any of the ages. For all the age groups, WBA was highest between 1500 and 5000 Hz and lowest at frequencies below 1500 and above 5000 Hz. Both the WBA of 0- and 6-month-old infants were significantly different to other age groups at most of the frequencies. The infants aged 0 to 2 months demonstrated a multi-peaked pattern while the 4- and 6-month-olds exhibited a single broad-peaked pattern. There was a significant difference in WBA across most frequencies between 0- and 6-month-old infants. Therefore, the null hypothesis (H_0) that predicted that there will be no significant age effects on WBA results obtained from infants aged 0- to six months was rejected. Although the WBA patterns were the same between the present study and those reported in the literature, there were differences in magnitude of WBA across the studies that were attributed to differences in subject sampling, equipment, test conditions and reference standards for middle ear function

The significant changes in WBA with age were suggestive of maturational effects in the first six months of development. These developmental changes may be attributed to changes in bone density of the ossicles and loss of mesenchyme and fluids in the middle ear, resulting in a smaller mass and resistive component of the middle ear. Normative data provided in the study (Chapter Six - Appendix A) could be used as a reference for detecting disorders of the conductive pathway in young infants.

7.6 Implications for Clinical Practice

7.6.1 Application of WBA as an adjunct tool during NHS

Based on the results of the study, WBA appears to be a valid tool of middle ear evaluation that shows promise as an adjunct device in a NHS program. This recommendation is based on the following research findings: (1) WBA had higher test performance against composite test batteries compared to single test reference standards in identifying disorders of the conductive system (see Chapter 4), (2) WBA was significantly lower in ears that failed either individual tests or test batteries compared to ears that passed (see Chapter 4). (3) WBA of ears that failed test battery reference standards were below the normative range across most of the frequency range between 250 and 8000 Hz (see Chapter 4), and (4) WBA identified Aboriginal infants with a significant middle ear condition that was not identified by a battery of screening tests (see Chapter 5).

WBA could be used as an adjunct tool to reduce false positive referrals as well as prioritise infants referred for diagnostic evaluation through a NHS program. By incorporating a set of WBA pass criteria into the equipment, an automated response (pass or refer result) could be displayed similar to that used in automated OAE or AABR devices. Since the results do not need to be analysed by the screening staff, the WBA equipment could be employed by the screening personnel in conjunction with other NHS tools.

A model for incorporating WBA into UNHS is provided in Figure 7.1. According to this model, infants who do not pass their first AABR/OAE screening would be rescreened, preferably before discharge from the hospital. If the infants do not pass their second screening in one or both ears, WBA would be administered. Neonates who receive a refer result during second AABR/OAE screening but have normal WBA (i.e., normal outer/middle ear function), would be considered to have a high risk of permanent hearing loss. Hence these neonates would be referred to an audiologist immediately and diagnostic evaluation recommended within two weeks. Neonates with a refer result in both WBA and second screening would receive a third AABR/ OAE screening within four to six weeks time. If they receive a refer result in the third screening, further diagnostic audiology evaluation would be recommended. This proposed model could reduce the referral rates for diagnostic audiology as well as assist in prioritising infants for diagnostic assessment. Use of WBA as an adjunct

tool following second AABR/OAE screen could determine if a conductive condition exists in the presence of a refer result.

Neonates with sensorineural hearing loss would be identified when normal WBA results are obtained along with a refer result on second AABR/OAE screen. Alternately, neonates with refer for both second screen and WBA, are likely to have a conductive condition. These neonates should receive a third AABR/OAE screen between four and six weeks' time. This time frame is recommended for the third screen, because, as described in Chapter 2, most conductive conditions in infants were reported to be resolved by four weeks following screening. Therefore, the majority of infants would pass their third screen due to resolution of their conductive condition. Those infants who refer in their third screen are likely to have either conductive or mixed hearing loss and therefore require further diagnostic assessments.

Studies by Boshuizen et al. (2001) and Dalzell, Orlando and Seeger (1996) recommend a three-stage screening model for NHS since it reduces the referral rate and costs less than a two stage process because of the lower cost of diagnostic facilities. The proposed model with addition of WBA as an adjunct tool to a three-stage screening model has further advantages. First, false positive referral rate could be reduced further with the use of WBA as an adjunct tool, since only neonates with hearing loss would be referred for diagnostic assessment. During the second screening, infants with risk of sensorineural hearing loss would be referred while during the third screening, infants with persisting conductive and mixed hearing loss would be identified. The majority of infants whose hearing returns to normal with the resolution of middle ear function would not be referred for diagnostic evaluation. As shown in Chapter 2, about 55% of infants had normal hearing during their initial diagnostic evaluation. Thus, the false positive referral rate could be reduced by more than half with the use of WBA as an adjunct test.

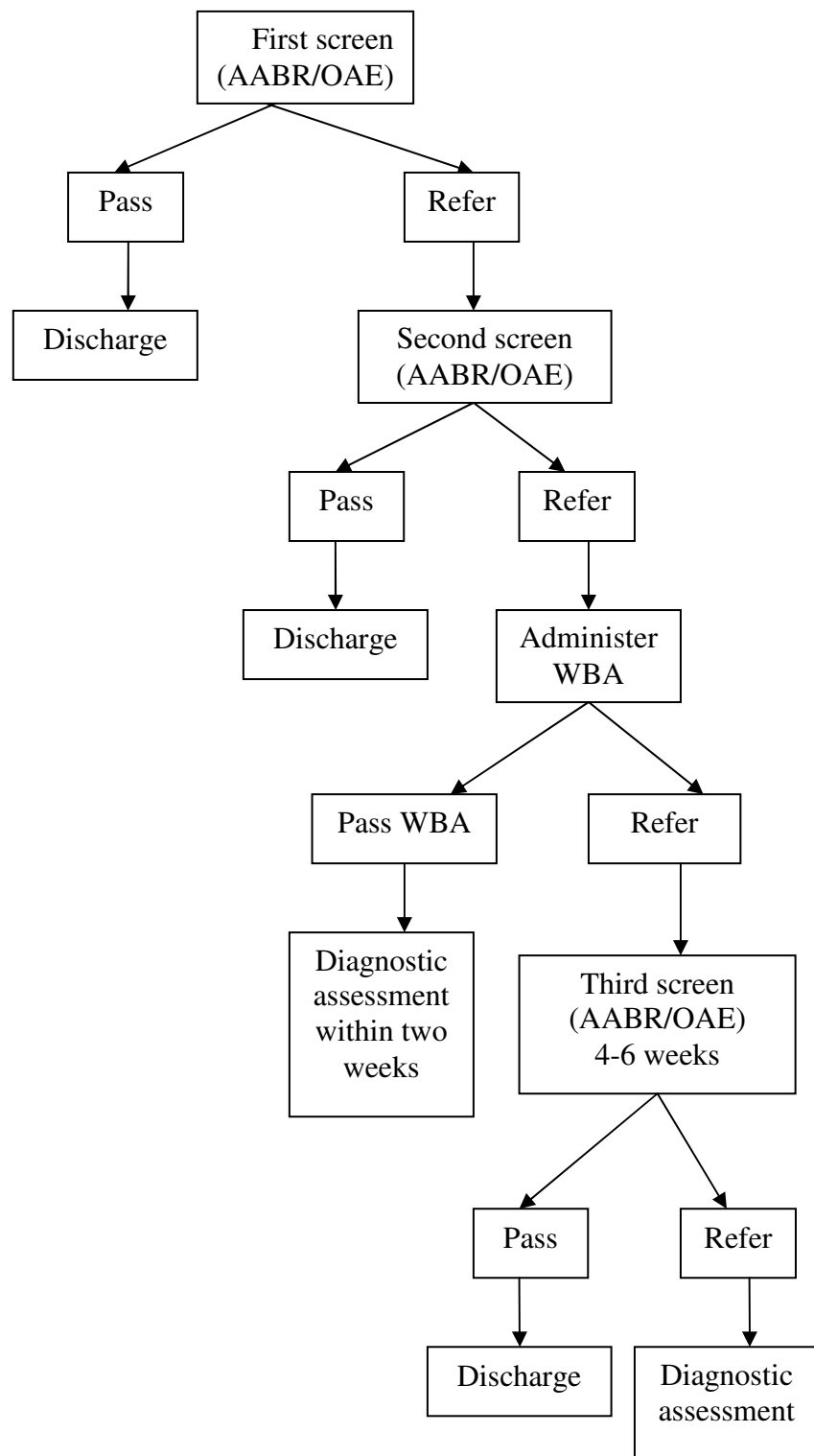


Figure 7.1: Proposed model for NHS with WBA as an adjunct tool for assessment of middle ear

Second, neonates with high risk of sensorineural hearing loss would be prioritised for diagnostic assessment. This would ensure early diagnosis of sensorineural hearing loss. Since diagnostic evaluation is recommended within two weeks' time, there would be no delay in the diagnosis of hearing loss in these infants. Third, the follow up of infants at risk of sensorineural hearing loss could also be prioritised. For instance, the follow up of those neonates who do not pass second screening but pass WBA and miss their diagnostic appointment, could be prioritised for follow up due to their high risk for sensorineural hearing loss. Health resources could be better utilised to target these infants to ensure their attendance for follow up so that they receive timely diagnostic evaluation and early intervention. This could reduce neonates lost to follow up and improve the efficacy of the program. Fourth, the use of WBA could provide additional information about the middle ear status, and, therefore, reduce the number of instances where ambiguous results are obtained during diagnostic evaluation. This would reduce the need for multiple reviews for these infants, and, again ensure early identification of hearing loss without delay. This would reduce unnecessary cost for those families who need to travel long distances with young infants for diagnostic assessments.

7.6.2 Application of WBA during diagnostic evaluation of neonates and infants

7.6.2.1 WBA during diagnostic evaluation of neonates

In view of its superior performance against composite tests compared to single test reference standards, WBA shows promise as a clinical tool for assessing the conductive pathway in neonates (see Chapter 4). WBA could be used as a single clinical tool with test performance which is as good as the test battery reference standards.

The present study obtained normative data using a robust reference standard for middle ear function and defined the normative range for WBA as between the 10th and 90th percentiles (see Chapter 2). These normative WBA measures could be used clinically to determine the status of the conductive mechanism in neonates. The WBA of neonates could be plotted against this normative range. Neonates with WBA values falling within this normative range are regarded as having an effective conductive pathway, while those with WBA falling outside this range would require further

medical investigation. This is also supported by the finding wherein WBA was significantly lower for ears with middle ear dysfunction compared to ears with a normal conductive pathway.

The present study found the frequency region of WBA between 1000 and 2500 Hz to have the highest discriminability for conductive conditions. This is consistent with the results of other studies (Hunter et al., 2010; Sanford et al., 2009). Therefore, greater weight should be apportioned to this frequency region in clinical assessments of neonates.

7.6.2.2 WBA during diagnostic evaluation of young infants

The present study provided normative data for infants at 0, 1, 2, 4 and 6 months of age (chapter 6). These data could be used as a reference for detecting disorders in the conductive pathway in young infants. Normative regions could be described as the region between the 10th and 90th percentiles, similar to that for neonates described in chapter 3. WBA of infants could be plotted against the normative data to determine if values fall within or outside of the normative range.

7.7 Limitations of the Investigation

In spite of the encouraging findings of this research, several limitations have been identified that could affect the clinical application of WBA. First, multiple tests were performed that involved inserting multiple probes into the ear canal of an infant. This procedure had the potential to disturb a well settled infant. This problem was partially overcome by testing the infants after feeding when they were well settled. Further research is recommended using equipment that allows all the tests to be done using a single probe.

Second, the test battery was time consuming. As the time for data collection was limited to certain hours of the day, all testing could not be completed in both ears of all infants recruited into the study. During the test, only the ear accessible easily was tested first and the second ear was only tested if time permitted and the infant was settled. This could have resulted in less number of ears that could be tested during any given testing session. Further studies could consider adapting flexible hours for data collection to allow data collection on a large number of infants.

Third, the sample size of the present study was small compared to the number of infants initially recruited. The present study has adopted a strict parallel protocol in which an ear was regarded as having a normal conductive pathway if and only if the ear passed all tests of the test battery. This strict protocol excluded the ears that passed one test but failed the others in a composite test reference standard, and therefore, reduced the sample size. The small sample size could have reduced the power of the statistical analyses especially in evaluating test performance of WBA and drawing conclusions regarding ethnic and gender differences in WBA. In order to overcome this, further studies need to consider methods to maximise recruitment of infants, such as, working flexible hours to collect data prior to discharge from hospital.

Fourth, the sample sizes were unequal during comparison of WBA between Aboriginal and Caucasian neonates (Chapter 5). The sample size for Aboriginal group was smaller than that of the Caucasian neonates and hence the finding of the study may not be confidently generalised. Further studies could incorporate larger sample of Aboriginal neonates and compare the test performance of WBA between Aboriginal and Caucasian neonates to draw conclusions regarding ethnic differences in outer/middle ear function.

Fifth, the results of the study could have been influenced by the lenient pass/fail criteria of some tests. For instance, the pass criterion for HFT was a single positive peak while double or multiple peaks were considered a fail. Similarly, the OAE pass criteria of at least a 3 dB SNR at 2000, 3000 and 4000 Hz may not be optimal for detecting conductive conditions, especially when the pass criteria did not assess frequencies below 2000 Hz where the effect of middle ear disorder may be more significant. Further studies could incorporate 1500 Hz into the protocol to investigate if the inclusion of these frequencies could improve the identification of conductive conditions.

Sixth, the time of testing can be a factor that influences test performance in neonates. Due to the possible clearing of vernix and mesenchyme in the first few days, the referral rates with tests such as HFT, OAE and WBA vary with the time of

testing. Sanford et al. (2009) reported a similar effect wherein the test performance of WBA in two-day-old infants was lower than that of one-day-old neonates. The mean age of neonates in the present study was higher than that of the other normative and test performance studies in neonates. Further studies should explore the time of testing further by assigning the infants into different assessment time groups and comparing the referral rates and the test performance between these groups.

Last, although the present study described developmental changes in the first six months of life, the use of a cross-sectional design could have, to some extent, restricted the investigation of maturation of the outer and middle ear of infants. To date, there is only one study by Shahnaz et al. (2014) that has followed the maturational changes in WBA in 18 infants from birth to six months of age. Future studies should employ a longitudinal design with larger sample size to more accurately track developmental changes in the conductive properties of the outer and middle ear of infants at shorter time intervals from birth to six months.

7.8 Conclusion

The present thesis explored the use of WBA in neonates and young infants. This research is the first to have used a combination of tests as a reference standard to develop normative WBA data and evaluate its test performance in healthy neonates. This research is also the first to have systematically compared the prevalence of conductive hearing loss and determined the outer and middle ear status of Australian Aboriginal and Caucasian neonates using WBA. This thesis has enhanced the minimal literature concerning the clinical application of WBA in young infants.

From the results of the series of studies described in this thesis, the following conclusions can be drawn:

1. Conductive hearing loss is a common diagnosis among infants referred through NHS in Australia. This finding is similar to that reported by NHS programs in other countries.
2. In view of the prevailing conductive hearing loss in neonates, an adjunct test of the outer and middle ear is recommended at the time of screening to

determine the status of the conductive pathway and prioritise the infants for diagnostic assessment.

3. Australian Aboriginal infants referred from screening have a higher prevalence of conductive hearing loss at birth and show poor resolution of middle ear dysfunction over time.
4. Use of a test battery to develop normative WBA data in neonates constitutes a new reference standard for the determination of outer and middle ear status. This composite tests reference standard represents the best available reference standard since invasive procedures such as myringotomy cannot be used in healthy neonates
5. WBA is a valid and sensitive test of outer and middle ear function in neonates. It has great promise to be used as a diagnostic test in paediatric clinics.
6. Furthermore, WBA could be used as a single clinical tool with high test performance which is as good as that of the composite tests reference standards.
7. Australian Aboriginal neonates have a subtle conductive condition that may not be detected by a battery of screening tests including HFT and DPOAE, but identified by WBA which is sensitive to outer and middle ear dysfunction.
8. Maturation of the outer and middle ear of infants in the first six months of life could be demonstrated using WBA. The results showed changes in WBA with age during this fast developing period.

7.9 Directions for Future Research

Although WBA has shown great promise in the determination of middle ear function in young infants, further research is warranted prior to its implementation as an adjunct screening tool in NHS programs or as a diagnostic instrument in clinics. For example, it would be ideal to evaluate the test performance of WBA against an ideal gold standard to determine middle ear status in neonates. In the absence of an ideal gold standard, the use of a surrogate gold standard such as the test battery standard or air/bone conduction ABR may be a viable alternative. To date, only one study has determined the accuracy of WBA using air and bone conduction ABR in young infants aged three to 26 weeks (Prieve et al., 2013). Further large scale studies

incorporating air and bone conduction ABR as the gold standard for conductive status would contribute to improvements in the precision of WBA normative data for neonates and young infants.

The WBA pass/fail criteria were based on the normative range for each frequency or limited range of frequencies (Chapter 2). However, such criteria for interpretation of WBA results are not quick enough to be used in a clinical setting. For optimal use in the clinical context, fast, efficient and objective methods for interpreting WBA findings need to be developed. This is especially important if WBA is to be used as an adjunct tool in NHS programs. Objective pass/fail criteria could be developed and built into the equipment so that a pass or refer result could be visually displayed on the screen similar to that used for AABR testing.

Persistent MEE in the neonatal period is reported to be a precursor for middle ear infections later in life. The present study has shown that many Aboriginal neonates have a significant conductive condition at birth. Further longitudinal studies are needed to establish the natural history and resolution of conductive disorders such as MEE in early infancy in both Aboriginal and Caucasian neonates to determine whether early MEE is a precursor for later persistent middle ear infections. Such studies would be useful in determining if targeted monitoring of infants at a high risk of recurrent OM is required. Additionally, it would also be helpful to develop appropriate public education programs and management strategies for infants at risk of developing recurrent OM.

Although Aboriginal neonates have been found to have lower WBA than Caucasian neonates, the reasons for the difference are not known. To date, there are no studies that have investigated anatomical and physiological differences in the outer and middle ear between Aboriginal and Caucasian neonates. Further research is needed to investigate the effects of body size on WBA in Aboriginal and Caucasian neonates and compare the findings with other ethnic groups with significantly different body size indices. Additional research using radiological evidence in infants is also required to investigate if a difference in the volume of middle ear cavity is associated with difference in WBA between the two groups.

The present study found developmental changes in WBA in the first six months of life. As development of the outer and middle ear continues beyond six months, further research is needed to establish normative WBA at close age intervals up to three years of age to provide additional maturation information. Such age appropriate norms are also necessary to differentiate normal ears from ears with a disorder of the conductive pathway. In addition to the developmental changes described using the cross-sectional sample in this study, further WBA research is needed to investigate individual differences in the developmental time course of the outer and middle ear.

WBA can be measured under ambient or pressurised conditions. Most often, studies have investigated middle ear function and maturational changes in the middle ear of infants using WBA under ambient pressure conditions. There is less understanding regarding the effects of introducing ear canal pressure in neonates (Feeney and Sanford, 2012). In comparison, wideband tympanometry (WBT) that involves measurement of WBA under conditions of varying ear canal pressure has been found to be useful in the determination of middle ear condition in adults. In view of the need for improved assessment techniques of middle ear function in neonates and young infants, application of WBT should be further explored in neonates and young infants. Further research is needed to develop fast and efficient methods of interpretation of results and identify predictive variables for determination of the middle ear condition using WBT.

In essence, the present study demonstrated that WBA is a feasible measure of middle ear function in neonates and young infants. Future investigations of WBA need to focus on determining the potential value of various WAI measures in large samples of infants. Objective, automated protocols developed using these measures could be used during screening and diagnostic evaluation of infants. The current thesis has shown direction in this undertaking by providing data for better interpretation of middle ear status in neonates and young infants.

7.10 References (For introduction, review of literature, introductions for chapters 2 to 6 and general discussion)

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